Errata Sheet

Causes and Consequences of Algal Blooms in the Tidal Freshwater James River, Submitted to The Virginia Department of Environmental Quality Agency by Joe Wood, Paul Bukaveckas, Greg Garman & Stephen McIninch, Center for Environmental Studies, Virginia Commonwealth University, April 2013.

DEQ considers all chlorophyll data to be provisional. Those data collected prior to 2014 were generated using analytical methods not certified through the Virginia Environmental Laboratory Accreditation Program (VELAP) as required by Regulations of the Dept. of General Services (1VAC30-45 & 1VAC30-46). Any use of those data shall be considered provisional pending results of a comparison study between method used prior to that date and VELAP methods.

Causes and Consequences of Algal Blooms in the Tidal Freshwater James River

Joe Wood, Paul Bukaveckas[1], Greg Garman & Stephen McIninch
Center for Environmental Studies
Virginia Commonwealth University

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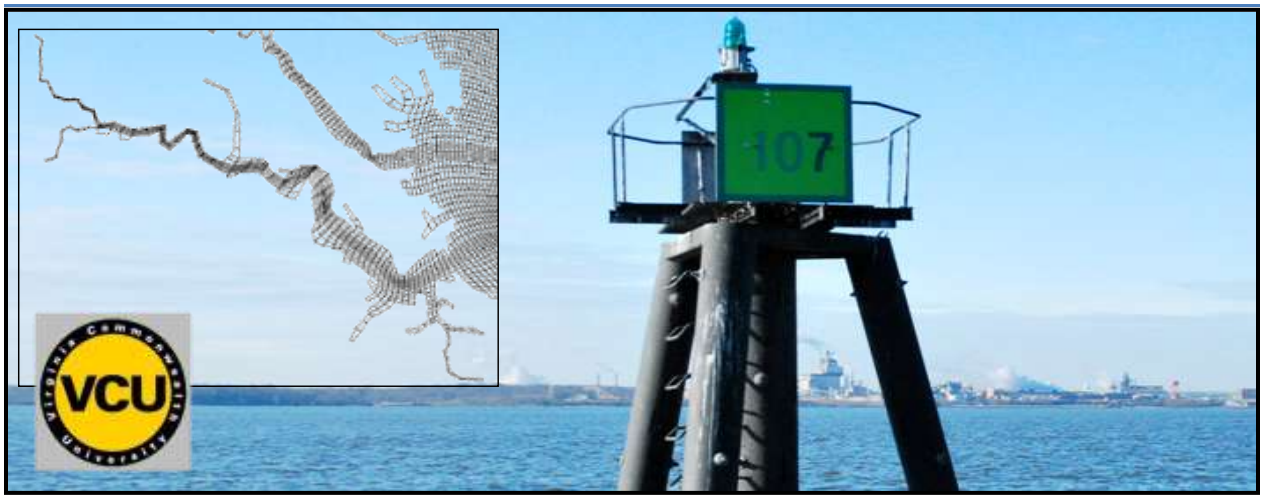
1. Author to whom correspondence should be addressed (pabukaveckas@vcu.edu)



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Background

Tidal freshwaters occur at the interface between riverine and estuarine environments and are characterized by the presence of bi-directional tidal forces in the absence of salinity (<0.5 ppt). Their presence is a common feature in estuaries along the US Atlantic and Gulf coasts, as well as in Europe and the southern hemisphere (Baldwin et al. 2009). These systems typically experience high nutrient loads due to their proximity to riverine inputs and, in some cases, from point sources in urbanized coastal areas. Tidal freshwaters are not well-studied in comparison to saline estuaries, but are known to be highly productive. The tidal freshwater segment of the James River exhibits high phytoplankton production and among the highest annual average chlorophyll-a concentrations (CHLa) throughout Chesapeake Bay and its tributaries (Figure 1).

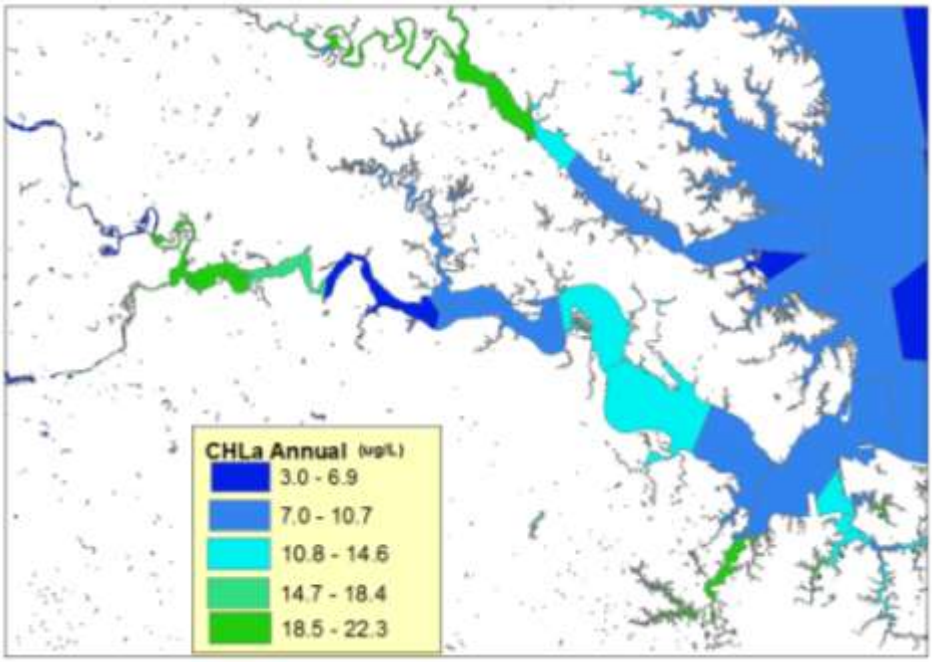


Figure 1. Annual average CHLa concentrations in the James and York River Estuaries during 2005-2010 (data from CBP).

The James is designated an impaired waterway because chlorophyll concentrations exceed numeric CHLa standards (DEQ 305(b)/303(d) Integrated Report). High phytoplankton production in this region has been attributed to favorable light and water residence conditions where the channel transitions from a riverine (deep, narrow) morphometry to a wider channel with extensive shallow areas (Bukaveckas et al. 2011). Shallow depths result in higher light intensities within the water column resulting in greater nutrient utilization by phytoplankton.

In order to improve our understanding of nutrient effects on tidal freshwaters, it is critical to assess factors controlling phytoplankton abundance. Resources that regulate phytoplankton growth include mineral nutrients (N & P) and co-variables such as light intensity and water residence time. Key questions to be addressed are: (a) Under what conditions are phytoplankton growth rates constrained by mineral nutrients? (b) Which nutrients are limiting (N vs. P)? and (c) To what extent are different forms of N (NO_3, NH_4 & DON) utilized by phytoplankton?. At present there is little information for the tidal freshwater segment of the James as to the occurrence of N vs. P limitation and the utilization of various forms of N. Prior studies have typically measured light-saturated rates of nutrient utilization (Fisher et al. 1999) which exceed

in situ irradiance and therefore are indicative of potential, not actual, limitation (Bukaveckas et al. 2011). Resolution of these issues has implications for modeling bloom development, which is needed to link nutrient loads to CHLa, and may also be important to guiding nutrient mitigation strategies. Analysis of N and P availability in the James suggested that N limitation is likely during the early-summer bloom initiation period, but that P limitation may occur in late summer when cyanobacteria dominate. N limitation is expected to be more severe in near-shore areas where DIN concentrations are lower. In addition to "bottom up" controls, phytoplankton may also be constrained by "top down" (grazing) effects. Potential consumers of phytoplankton include a wide range of benthic and pelagic organisms such as zooplankton, macroinvertebrates and fish. Identifying important grazer pathways and quantifying rates of CHLa removal is central to modeling efforts to forecast CHLa under various nutrient loading scenarios.

Anthropogenic nutrient loads stimulate phytoplankton production and result in an array of degrading effects on aquatic ecosystems. The incidence of harmful algal blooms has been increasing worldwide (O'Neil et al. 2012). In fresh waters, blooms of cyanobacteria, and particularly those which have the ability to produce toxins, are of special concern. These toxins represent a threat to drinking water supplies, human recreational activities and living resources (Poste et al. 2011). In order to assess the impacts of anthropogenic nutrient loads, it is necessary to document the occurrence of cyanobacterial blooms and the spread of cyanotoxins through the food web.

The Commonwealth of Virginia initiated a multi-year study of the James to better understand the causes and consequences of algal blooms and to develop predictive models linking CHLa to nutrient inputs. This report presents the results of research activities undertaken in the tidal-freshwater segment by Virginia Commonwealth University (VCU) during 2012. Specific objectives were (1) determining physiochemical limitations on phytoplankton growth by measuring their responses to light and nutrient manipulations, (2) characterizing top-down controls of phytoplankton abundance by identifying important grazers and quantifying their effects and (3) assessing cyanobacteria blooms by measuring the toxin Microcystin in water, sediment and biota. Results from this research support efforts to evaluate existing CHLa criteria for the James and to further refine the associated modeling framework for assessing attainability under various nutrient management scenarios.

Study Site

The James River is the third largest tributary of the Chesapeake Bay by discharge and nutrient load. The Tidal Fresh James extends 58 km from the fall line in Richmond, VA to the confluence with the Chickahominy River below Hopewell, VA. This portion of the river, though small in surface area, receives substantial nutrient loads from a large watershed (26,165 km^2) as well as local point sources from the Richmond Metropolitan Area (Bukaveckas and Isenberg, in review). The summer phytoplankton community is dominated by cyanobacteria which have been increasing in abundance over the past 30 years (Marshall et al. 2008). Spatial and temporal dynamics of algal blooms in this region are well characterized by long-term (since 1985) monthly monitoring by DEQ-CBP and weekly monitoring (since 2010) carried out by VCU. Sampling for research activities described in this report was conducted within the tidal freshwater segment (Figure 2). Monitoring efforts were centered on long-term CBP sites which are referenced according to their DEQ designations (JMS75, etc.).

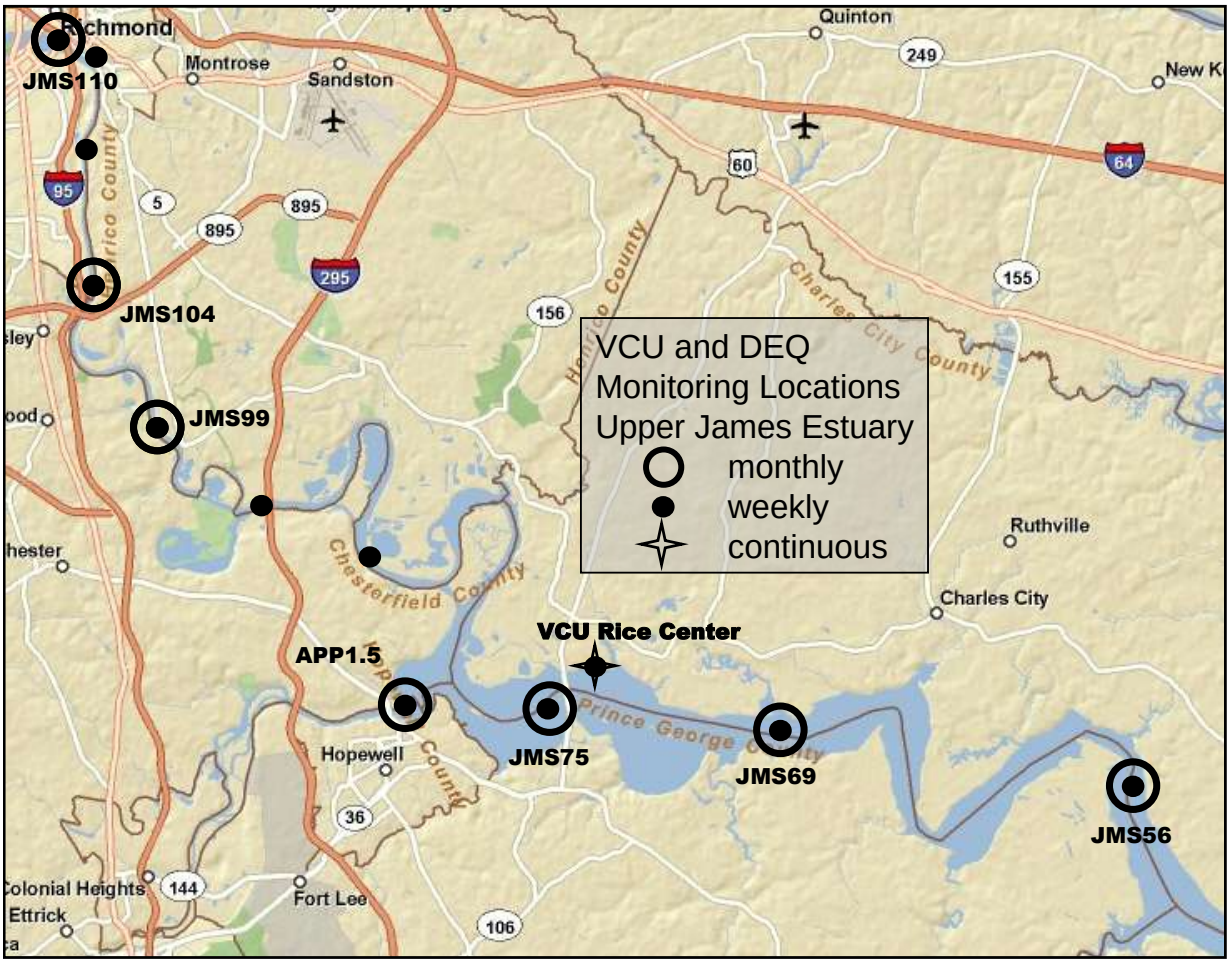


Figure 2. Map of tidal freshwater James River showing 2012 sampling locations for CHLa and nutrients. Monthly sites are sampled by DEQ for CBP; weekly sites are sampled by VCU. A continuous water quality monitoring station with bi-weekly sampling is operated by VCU.

Part 1: Nutrient and Light limitation of Phytoplankton Production

Introduction

Alleviating impacts of eutrophication requires an understanding of phytoplankton resource limitation (Elser et al. 2007). Phytoplankton shift between nutrient and light limitation throughout the year as a result of seasonal patterns in solar radiation as well as variable nutrient delivery associated with seasonal patterns in runoff. Resource dynamics in tidal freshwater estuaries are further complicated by the interplay between riverine and tidal source waters which differ in their nutrient and sediment loads. Light availability has a direct effect on phytoplankton growth rates; however few studies analyze nutrient limitation in the context of realistic underwater irradiances. Older studies generally measured nutrient effects at saturating light levels, whereas more recent studies align experimental light conditions with in situ light conditions (e.g., Koch et al. 2004; Whalen and Bensen 2007). This approach provides a basis for assessing phytoplankton nutrient limitation at meaningful light levels and for describing interactive effects of light and nutrients on phytoplankton growth rates.

Nutrient limitation arises from low supply to demand ratios and typically occurs during the summer, warm-water period when algal biomass is highest and watershed runoff is at a minimum. Nutrient supply is governed by external loading and internal recycling. P loads to the tidal freshwater James are principally (~80%) from watershed sources which are transported in particulate form during high discharge events (Bukaveckas & Isenberg, in review). For N, watershed and local point sources contribute approximately equally, though the latter dominate with respect to dissolved inorganic fractions and during low discharge periods. Historically, freshwater environments were categorized as P-limited due to their proximity to N-rich point sources and because N-fixation by cyanobacteria offset N limitation. Conversely, marine environments were thought to be N-limited due to high rates of denitrification in coastal sediments (Howarth 1988, Seitzinger 1988, Vitousek and Howarth 1991). More recently it has been recognized that most aquatic systems experience co-limitation by N and P as well as seasonal shifts in their relative importance (Elser et al. 2007). Understanding of nutrient limitation is further complicated by the presence of various forms of N and P which may differ in their bioavailability. For N, uptake of dissolved inorganic forms (e.g., nitrate and ammonia) is well-known, whereas utilization of dissolved organic nitrogen has only recently been appreciated (Mulholland et al. 2009, Filippino et al. 2011).

In dynamic systems such as estuaries, resource limitation may be transient in nature as phytoplankton shift between light and nutrients, and among various forms of nutrients, in response to seasonal and episodic events that influence nutrient delivery and light attenuation. In theory, elemental limitation may be inferred by comparing nutrient availability (as concentrations in the environment) to phytoplankton nutrient demand (inferred from stoichiometry; Redfield 1958, Ptacnik et al. 2010). This approach relies on the assumption that measured nutrient concentrations reflect availability which may be problematic given differences in lability among various nutrient fractions (Beardall 2001). Determining limitation through bioassay experiments, which directly measure growth responses to nutrient amendments, is an alternative approach which allows nutrient uptake rates and biomass production to be directly measured (Tamminen and Andersen 2007, Ren et al. 2009). In this study, we used bioassay experiments to: (1) characterize seasonal patterns of nutrient and light limitation, (2) determine

which elements (N vs. P) limited phytoplankton growth, and (3) identify which forms of nitrogen were used by phytoplankton.

Hypotheses

Nutrient concentrations and freshwater turnover times for the tidal freshwater James River were used to develop hypotheses regarding seasonal patterns in resource limitation. Inorganic nutrient concentrations typically decline during the growing season due to increased assimilation. Simultaneously increasing water residence times result in greater phytoplankton biomass and nutrient demand. Based on these trends, we hypothesized that phytoplankton nutrient limitation would be most severe in late summer (H1). This hypothesis was tested by measuring phytoplankton growth rates under ambient and nutrient-enriched conditions over the growing season (May-October). A second hypothesis was developed focusing on spatial variation in nutrient limitation. While the James is generally a well-mixed system due to the large tidal amplitude, our prior data have shown lateral variation in DIN with lower concentrations in near shore sites (P. Bukaveckas, unpubl.. These shallow areas may be more DIN-depleted due to an increased capacity for sediment de-nitrification to influence water column concentrations. We hypothesized that near shore sites will be more likely to experience nitrogen limitation (H2). This hypothesis was tested by comparing phytoplankton growth rates under ambient and N-enriched conditions using water collected from the main channel and a near-shore site (Rice Pier). A third hypothesis was developed regarding N vs. P limitation based on observed nutrient concentrations in the James and recently published limitation thresholds (Ptacnik et al. 2010). DIN:TP ratios were found to be seasonally variable with values suggestive of N limitation during June, July and August (Figure 3). Based on these values, we hypothesized that phytoplankton will be principally

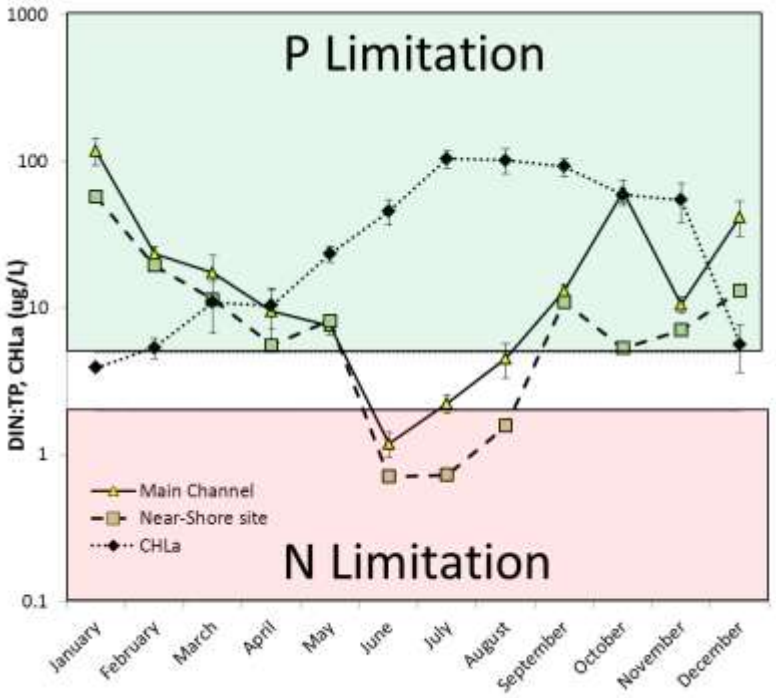


Figure 3. Seasonal variations in DIN:TP for the tidal fresh James River near shore (VCU Rice Pier) and main channel (JMS 75) sites. Prediction thresholds indicate values specified by Ptacknik et al. 2010.

limited by the availability of N (H3). This hypothesis will be tested by comparing phytoplankton growth rates of N- vs. P- enriched bioassay cultures during May-October. We also considered the role of various forms of N in supporting phytoplankton production as the James receives inputs of NO_3, NH_4 and DON from both diffuse and local point sources. We hypothesized that the inorganic forms would be utilized by phytoplankton more readily and therefore support higher biomass yield (H4). This hypothesis was tested by comparing phytoplankton growth rates of NH_4, NO_3 and DON enriched bioassay cultures during May-October. Lastly, we considered the interactive effects of light and nutrients on algal growth rates. Prior work on this segment of the James suggested that changes in channel morphometry release phytoplankton from light limitation and allow for greater nutrient utilization (Bukaveckas et al. 2011). We tested this hypothesis (H5)by comparing phytoplankton growth rates of ambient and nutrient (+PN) enriched bioassay cultures at light levels corresponding to those occurring in the region of the CHLa maximum and the upper, deeper channel where CHLa was low.

Methods

Algal bioassay experiments were performed monthly from May to October 2012 using river water obtained from JMS 75 (main channel) and the VCU Rice Pier (near shore). Water collected for these experiments was obtained in conjunction with a ~weekly river monitoring program carried out by VCU. The monitoring program characterizes longitudinal variation in CHLa, nutrients and water quality at 11 stations in the tidal freshwater segment. Bioassay cultures comprised a 150 mL solution in a 250 mL Erlenmeyer flask containing 50% filtered river water and 50% raw river water. Cultures were diluted to reduce algal densities below equilibrium and allow measurement of algal growth responses. For water collected from the Rice Pier, 6 treatments of 3 replicates each were carried out: Ambient, $+NH_4$, $+NO_3$, + Urea, +P, +PN (Table 1). Only ambient and combined (+PN) treatments were performed on main channel cultures.

Table 1. Experimental Design for bioassay experiments performed on phytoplankton communities from the tidal fresh James River during 2012.

Nutrient Treatment	High Light (12 E m^{-2} d^{-1})		Medium Light (6 E m^{-2} d^{-1})		Low Light (3 E m^{-2} d^{-1})	
	Near-shore	Main Channel	Near-shore	Main Channel	Near-shore	Main Channel
Control	x3	x3	x3	x3	x3	x3
$+NO_3$	x3	-	-	-	-	-
$+NH_4$	x3	-	-	-	-	-
+Urea	x3	-	-	-	-	-
+P	x3	-	-	-	-	-
+PN	x3	x3	x3	x3	x3	x3

Nitrogen enrichments raised the concentrations of NO_3, NH_4, and Urea by 0.125 mg/L and P enrichments raised the concentration of PO_4 by 0.1 mg/L. We chose a higher rate of P enrichment because PO_4 is considered a very conservative estimate of P availability. The Combined treatment (+PN) included 0.125 mg/L each of NO_3 and NH_4 (total = 0.250 mg DIN/L) and 0.1 mg PO_4 /L. These additions approximately doubled the ambient concentrations increasing DIN from 0.10-15 mg/L to ~0.25-0.3 mg/L and SRP from 0.05-0.10 mg/L to ~0.20 mg/L. Cultures were incubated on a shaker table at 80 RPM inside a Conviron growth chamber for 48 hours at ambient (river) temperature. Cultures were subject to an irradiance of 12 $E/m^2/d$; this value represents the expected irradiance for the segment of the James near JMS75 (mean water column depth = 1.3 m), taking into account average daily solar radiation in this region (May-September = 40 $E/m^2/d$; Fisher et al. 2003) and typical light attenuation (mean k_d = 2.67 m^{-1}; Bukaveckas et al. 2011). In order to assess light limitation and interactive effects of light and nutrients, additional replicates of the Control (ambient) and Combined (+PN) treatments were incubated at 6 and 3 $E/m^2/d$. These light intensities correspond to average water column depths of 3.5 and 5.5 m, respectively, which are representative of the upper, deeper segment of James (e.g., JMS99). Various light levels were achieved by adjusting proximity to light sources and use of shading. Light levels within the growth chamber were verified using a Li-500 light meter. Light attenuation in the river was measured at the Rice Pier on the day of experiments using an underwater light sensor to confirm that previous estimates of k_d were representative.

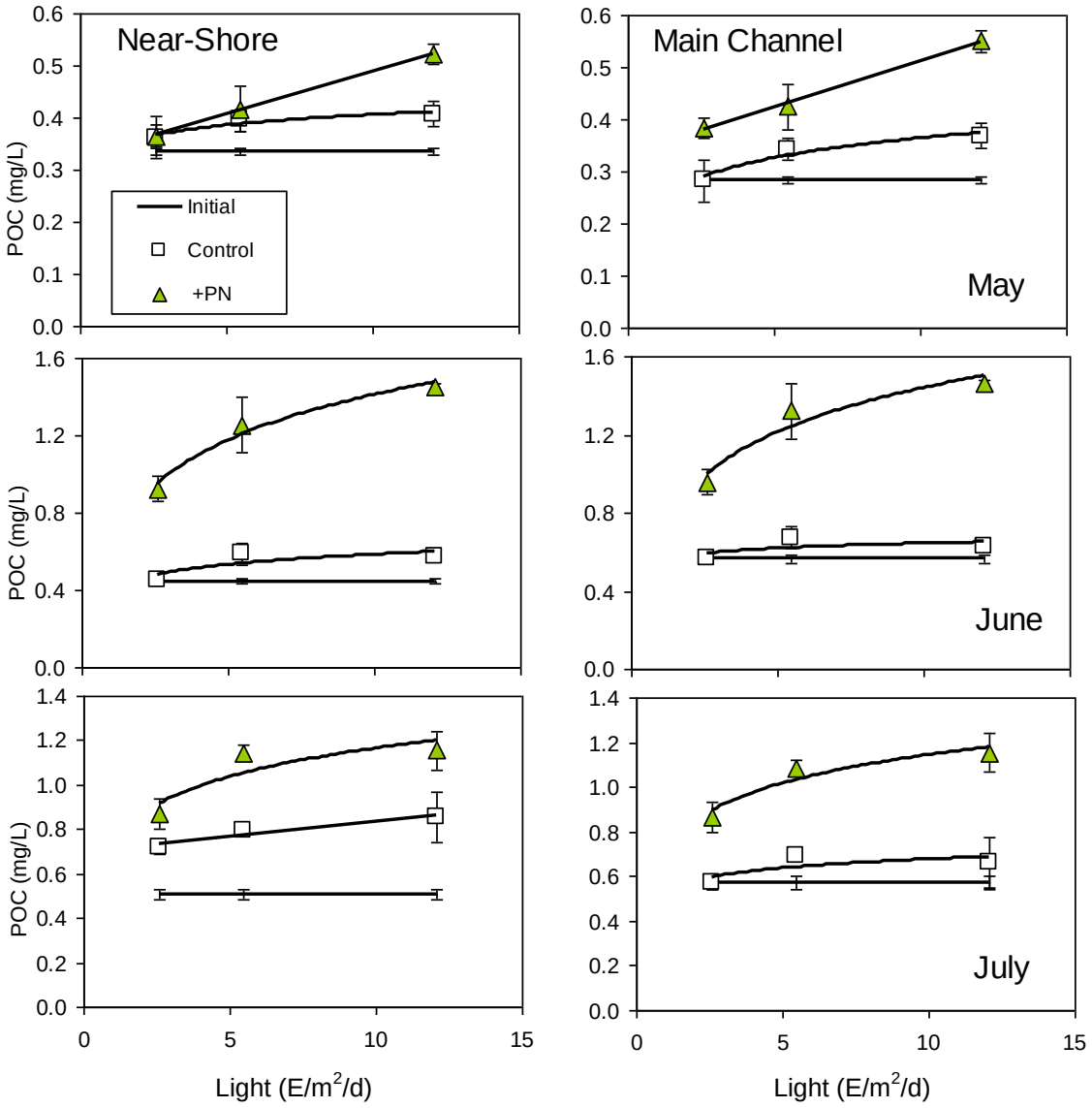
Following incubation, water was filtered through Whatman GF/A glass filters (0.5 μm nominal pore size). CHLa samples were extracted for 24 h in buffered acetone and analyzed on a Turner Design TD-700 Fluorometer. POC samples were dried at 60 C, exposed to acid fumes for 48 hours and analyzed on a Perkin–Elmer CHN analyzer. Concentrations of total nitrogen (TN), nitrate (NO_3) ammonium (NH_4), total phosphorus (TP) and phosphate (PO_4) were determined using a Skalar segmented flow analyzer using standard methods (APHA 1992). Urea concentrations in initial, ambient and urea-enriched were determined by the Mulholland lab at ODU. Water samples for microcystin analysis were collected and analyzed (see Methods, Part 3) to assess light and nutrient effects on toxin production.

Initial and final concentrations of CHLa, POC and nutrients were used to estimate phytoplankton growth rates and nutrient uptake. Growth rates (r) were calculated as the slope of the natural logarithms of POC as a function of time (Koch et al. 2004). Some other studies have used CHLa to calculate growth rates, however in preliminary experiments we observed large changes in POC:CHLa during incubations. POC-based growth rates were used to infer N, P, co-limitation or light limitation. ANOVA and ANCOVA were used to test for significant differences among sites (near-shore vs. main channel), forms of nutrient limitation (N, P, N+P), forms of N (NO_3, NH_4, Urea), and light levels. We used the ratio of ambient to nutrient-enriched growth rates as an index of the severity of nutrient limitation. Water residence time of the tidal freshwater segment was estimated as a freshwater replacement time based on measured discharge of the James and Appomattox Rivers.

Results

Phytoplankton exhibited positive responses to light gradients in each of the monthly experiments at both sites (Figure 4). Light effects on algal abundance were statistically significant in each month (Table 2). To assess light saturation effects, we tested linear and non-linear models; these provided a good fit to the data in all but one experiment (September, Near-shore site). In 5 of the 12 experiments, phytoplankton responses to increasing light levels were

non-linear (i.e., showing a saturation response). These findings suggest that phytoplankton growth rates at irradiances representative of the upper, constricted channel (3-6 E m^{-2} d^{-1};) were light-limited, whereas higher light conditions in the region of the CHLa maximum (12 E m^{-2} d^{-1}; JMS75) resulted in a lessening of the severity of light limitation. Nutrient additions resulted in statistically significant higher growth rates in 11 of the 12 experiments. Nutrient enrichment effects on growth rates were observed throughout the range of light intensities but larger responses were typically observed at the highest light intensity. Average effect sizes (ratio of enriched to ambient nutrient growth rates) were 0.26 ± 0.06, 0.31 ± 0.07 and 0.41 ± 0.07 at 3, 6, and 12 E m^{-2} d^{-1}, respectively (Table 2). Significant interaction effects were detected in 5 of 12 experiments indicating a non-additive effect from the combination of high light and nutrient enrichment. These findings support the hypothesis that shallow conditions in the region near Hopewell allow phytoplankton to more effectively utilize nutrient resources resulting in higher algal abundance. No statistically significant differences were observed in phytoplankton responses between the main channel and near-shore sites to either light or nutrient effects.



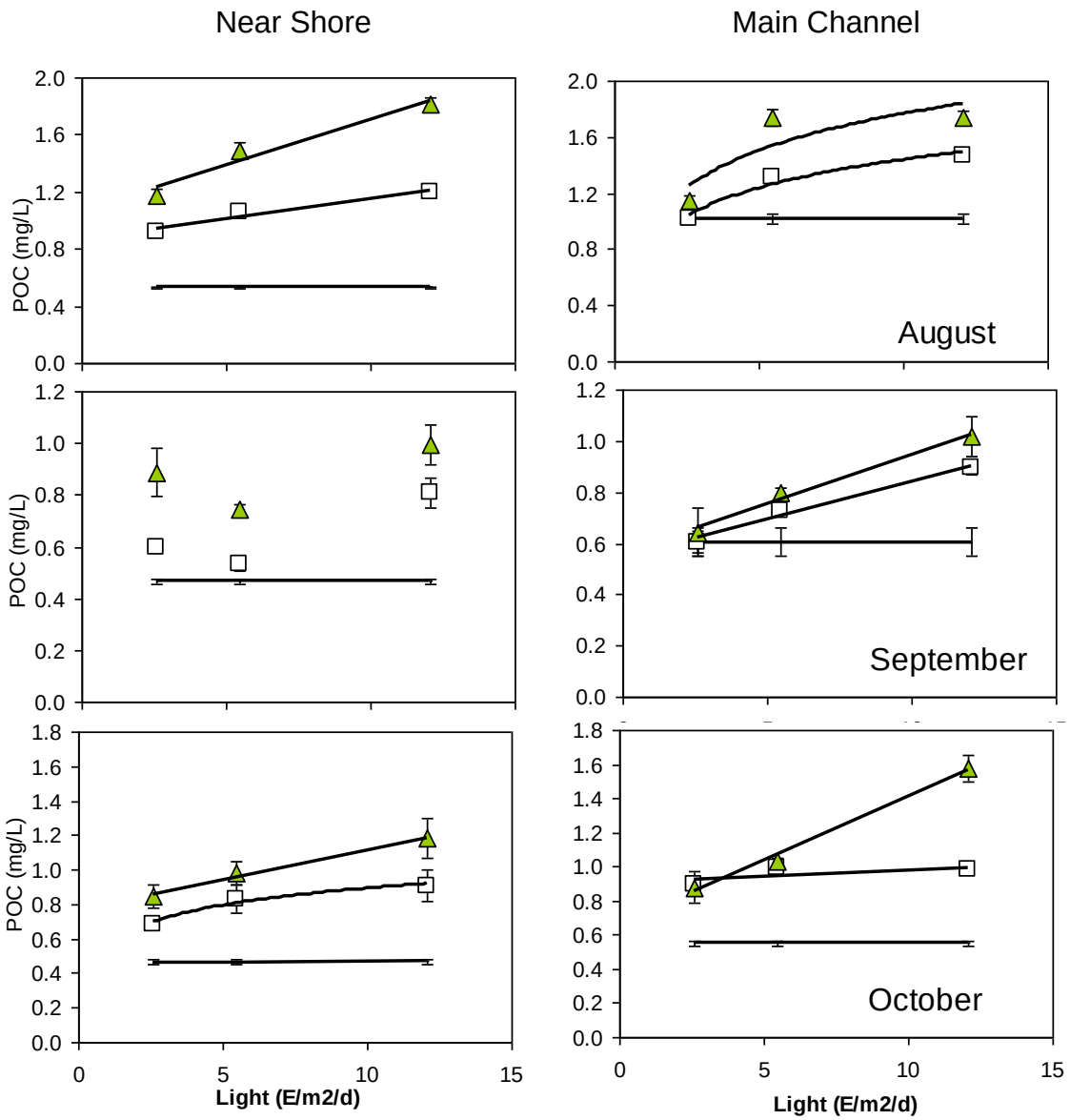


Figure 4. Algal responses (as POC) to light under ambient (control) and nutrient-enriched (+PN) conditions in bioassays performed at two stations in the tidal fresh James River (Near-shore = Rice Pier; Main Channel = JMS75). Error bars denote standard error (some not visible).

Site	Month	Nutrient Effect Sizes 3 E m^{-2} d^{-1}	6 E m^{-2} d^{-1}	12 E m^{-2} d^{-1}	Light vs. Nutrients (ANCOVA) Light (p)	Nutrients (p)	L * N (p)
Near Shore	May-12	0.01	0.05	0.25	0.003	0.045	0.036
	Jun-12	0.71	0.76	0.93	0.002	<.0001	0.024
	Jul-12	0.19	0.36	0.30	0.024	0.0006	*ns*
	Aug-12	0.25	0.34	0.41	<.0001	<.0001	0.003
	Sep-12	0.39	0.33	0.21	0.011	0.001	*ns*
	Oct-12	0.21	0.16	0.26	0.008	0.003	*ns*
Main Channel	May-12	0.30	0.21	0.40	<.0001	<.0001	*ns*
	Jun-12	0.53	0.67	0.85	0.003	<.0001	0.008
	Jul-12	0.42	0.45	0.55	0.017	<.0001	*ns*
	Aug-12	0.12	0.29	0.17	0.001	0.019	*ns*
	Sep-12	0.06	0.09	0.13	<.0001	*ns*	*ns*
	Oct-12	-0.02	0.04	0.48	<.0001	0.001	0.0003

Table 2. Effects of light and nutrient amendments on algal abundance (measured as POC) in monthly experiments performed at near-shore (Rice Pier) and Main Channel (JMS75) stations in the tidal fresh James River. L*N is the interaction between Light and Nutrient effects. Effect size is the natural-log transformed ratio of phytoplankton growth rates at enriched (+PN) vs. Control (ambient) nutrient concentrations.

Forms of nutrient limitation differed among the monthly experiments (Figure 5, Table 3). The combined P and N addition resulted in significantly higher growth rates relative to controls in all 6 of the monthly experiments. Higher growth rates in response to P addition were not observed in any of the experiments. Interpretation of N effects was somewhat dependent on the form of N tested. In June, all three forms of N (NO_3, NH_4 and Urea) resulted in significantly higher growth rates relative to Controls. Growth rates were not significantly different among the three treatments indicating that phytoplankton were capable of exploiting all three forms of N. In August, additions of NH_4 and Urea stimulated growth rates relative to Controls, whereas NO_3 did not. In September, cultures receiving NO_3, exhibited significantly higher growth rates relative to Controls and to those receiving NH_4 and Urea. Overall, these findings suggest that phytoplankton in the tidal freshwater segment of the James were responsive to additions of N alone, but that co-limitation by N and P was more common.

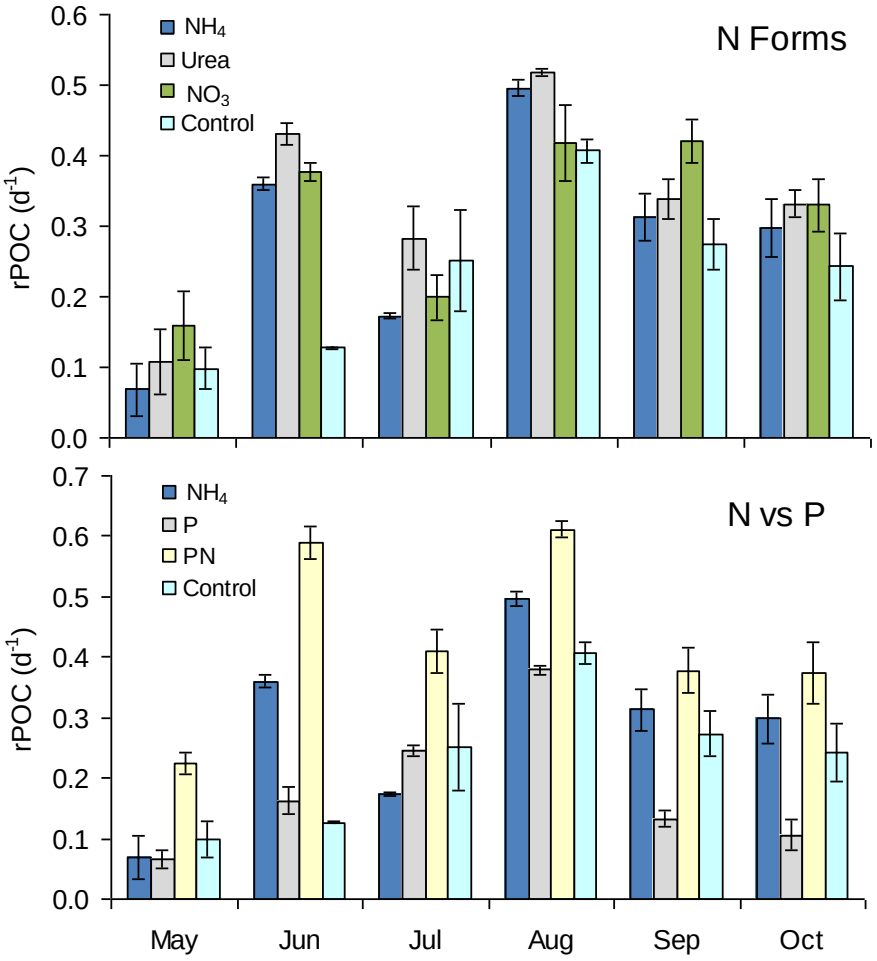


Figure 5. Mean phytoplankton growth rates (as C; ±SE) among experimental bioassays receiving various forms of N addition (top panel) and additions of N, P and P/N combined (lower panel) in comparison to Controls. Bioassay experiments were performed at a near-shore station (Rice Pier) located in the tidal freshwater James River.

	Nutrient Form growth rate comparisons (Tukey's HSD)				
	$+PO_4$	$+NH_4$	$+NO_3$	+Urea	+PN
Month	(p)	(p)	(p)	(p)	(p)
May-12	*ns*	*ns*	*ns*	*ns*	0.0003
Jun-12	*ns*	<.0001	<.0001	<.0001	<.0001
Jul-12	*ns*	*ns*	*ns*	*ns*	0.001
Aug-12	*ns*	0.04	*ns*	0.014	0.0003
Sep-12	*ns*	*ns*	0.004	ns	0.019
Oct-12	*ns*	*ns*	*ns*	*ns*	0.003
N	36	36	36	36	72

Table 3. Statistical analyses of C-based phytoplankton growth rates (rPOC) in bioassays receiving additions of P and N ('ns' denotes p>0.05). Samples sizes for the single nutrient additions are based on experiments performed at a single site (Rice Pier) with three replicates each for treatments and controls. The combined P and N addition was performed at two sites (including Main Channel – JMS75) and results were pooled for this analysis.

Phytoplankton growth rates at ambient and enriched nutrient concentrations were analyzed in relation to variation in nutrient availability and water residence time to assess factors influencing seasonal patterns in the severity of nutrient limitation (Figure 6). Growth rates at ambient nutrient concentrations ranged from 0.1 to 0.4 d^{-1} (mean = 0.23 d^{-1}) and corresponded to an average doubling time of 3 d. Seasonal variation in ambient growth rates followed patterns in CHLa (R^2 = 0.76 and 0.96 for main channel and near-shore sites, respectively) with peak growth rates corresponding to maximum CHLa in August (74 µg/L). Nutrient-saturated growth rates were higher (range = 0.2 to 0.6 d^{-1}; mean = 0.44 d^{-1}) and corresponded to an average doubling time of 1.6 d. Seasonal patterns in nutrient enriched growth rates were less apparent with lowest growth rates measured in May and highest rates in June and August. Stronger responses to nutrient enrichment were observed in May and June when ambient growth rates were <50% of nutrient-enriched growth rates. Weaker responses to nutrient enrichment were measured during July-October when ambient growth rates were ~60% of nutrient-enriched growth rates. Seasonal patterns in the severity of nutrient limitation followed trends in water residence time. Greater severity of nutrient limitation was associated with shorter water residence time in May June (5-10 d) with weaker responses to nutrient limitation occurring during periods of longer residence time (15-20 d).

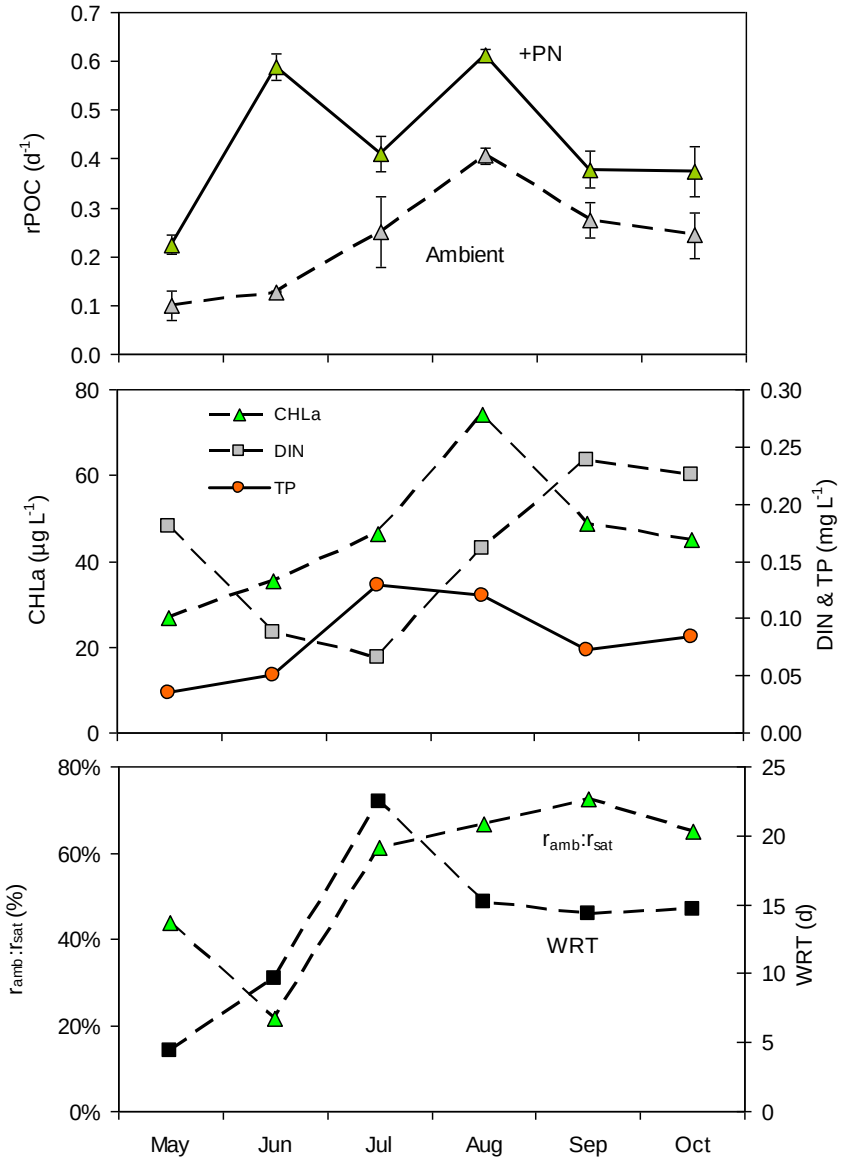


Figure 6. (Top) Phytoplankton growth rates (as C) at ambient and enriched (+PN) nutrient concentrations. (Middle) CHLa, DIN and TP concentrations in the tidal fresh James River. (Bottom) An index of the severity of nutrient limitation (ratio of ambient to saturated growth rates) and water residence time (WRT). Bioassay results and river data are for a near-shore station located at the VCU Rice Pier.

Discussion

Bioassay experiments were performed to test a series of hypotheses regarding the factors limiting phytoplankton production and their seasonal and spatial dynamics in the tidal fresh James (Table 4). The most striking finding was the positive response of phytoplankton to nutrient additions in 11 of 12 experiments, given that a prior study at this location reported no detectable response to nutrients among 11 experiments performed during 1989-1994 (Fisher et al. 1999). The prior study was conducted at the same location (JMS75 = TF5.5) but there are a number of methodological differences that complicate direct comparisons including use of different response variables (CHLa vs. POC), incubation length (6-8 d vs. 48 h) and light exposure (13-50 vs. 3-12 E m^{-2} d^{-1}; Fisher et al. 1999, this study; respectively). Two of these differences (lower irradiances and shorter incubations) would be expected to diminish the likelihood of observing nutrient limitation in our experiments and therefore it is unlikely that these methodological differences account for the contrasting results. One important aspect of methodology is experimental design which determines statistical power. The Fisher et al. study was a survey that encompassed much of Chesapeake Bay and therefore the effort allocated to a single site was small (e.g., based on two replicates for treatments and controls). By comparison, our treatments entailed three replicates, and for the combined nutrient addition, were tested at three light levels resulting in 18 degrees of freedom per experiment. One explanation for the difference in findings is that our experiments were more sensitive in detecting nutrient limitation effects due to their greater statistical power. We can not discount the possibility that changes in river condition during the 20-year interim between the studies could account for the observed differences, particularly if ambient nutrient concentrations have declined. Fisher et al. do not report nutrient levels associated with their experiments that would allow us to test this hypothesis directly.

Hypotheses	Outcome
(H1) phytoplankton nutrient limitation most severe in late summer	Not supported – greater nutrient limitation observed in early summer.
(H2) near shore sites more likely to experience nitrogen limitation	Not supported – no differences in severity of nutrient limitation observed between main channel and near-shore site.
(H3) phytoplankton principally limited by availability of N	Partially supported – though co-limitation was more prevalent
(H4) inorganic forms of N support higher biomass yield	Not supported – all three forms of N resulted in similar biomass yield.
(H5) changes in channel morphometry release phytoplankton from light limitation and allow for greater nutrient utilization	Supported – higher light intensities resulted in greater nutrient utilization and biomass yield.

Table 4. Summary of hypotheses and outcomes pertaining to algal bioassay experiments performed in tidal fresh James River.

Seasonal trends in nutrient limitation were opposite of expected patterns (H1) with strongest nutrient effects observed in early summer (May-August). Current paradigms on nutrient limitation are largely based on lake studies where increasing water residence time (WRT), coupled with thermal stratification, leads to progressive depletion of nutrients from the photic zone and greater nutrient stress in late summer. The relationships between WRT, nutrient supply and algal demand are more complicated in river-influenced environments where discharge exerts stronger effects on phytoplankton loss (through flushing) and growth (through nutrient delivery; Lucas et al. 2009). A recent study of the New and Neuse River estuaries revealed that CHLa was positively related to WRT at low WRT, but negatively related at long residence times (Peierls et al. 2012). The shift from positive to negative slope in the CHLa-WRT relationship was attributed to biotic processes which exerted a greater influence on phytoplankton abundance during long residence time. These included increases in the severity of nutrient limitation as well as higher losses due to grazing. In the James, positive responses to nutrient addition were observed during May-August indicating the presence of nutrient limitation despite comparatively short WRT (5-20 d). As elevated discharge is associated with higher nutrient loads, greater nutrient limitation during short WRT appears counter-intuitive. However, it is important to note that discharge is principally due to watershed runoff (point sources are small by volume) and that N yields from the James watershed are low among east coast rivers (Boyer et al. 2002; Howarth et al. 2006). Thus periods of elevated discharge are characterized by inputs of relatively dilute waters with respect to dissolved inorganic nutrients. Dissolved nutrient concentrations in point source inputs are orders of magnitude higher, and during low river discharge, these would be subject to smaller dilution effects. Thus the proposed mechanism to account for the observed seasonal patterns is that higher river discharge in early summer reduces nutrient concentrations in source waters to the estuary resulting in greater nutrient limitation. Reduced watershed runoff in late summer results in higher concentrations of inputs and weakens nutrient limitation. We cannot discount the possibility that shifts in phytoplankton community composition may contribute to seasonal patterns in nutrient limitation due to inter-specific differences in nutrient use efficiency. However, we note that nutrient-saturated growth rates did not exhibit consistent seasonal patterns as would be expected if late summer communities were more efficient in converting nutrients to biomass. Instead, seasonal patterns in the index of nutrient limitation were principally driven by changes in ambient growth rates, consistent with our hypothesis of greater nutrient availability in late summer, despite lower loading rates. An alternate explanation is that nutrient recycling accelerates in late summer thereby increasing nutrient supply and reducing nutrient limitation. Seasonal patterns in grazing by zooplankton (Bukaveckas et al. 2011) and benthic filter-feeders (see Part 2) do not support this view, though further work is needed to assess other pathways of nutrient supply (e.g., from sediments). An important implication of these findings is that nutrient limitation of phytoplankton in the tidal fresh James is principally determined by the concentration of nutrients in inflow sources, not the overall load. To further test this hypothesis, we analyzed variation in CHLa at JMS75 in relation to the concentration of DIN in inflow. The latter was calculated as a volume-weighted concentration taking into account weekly riverine and point source DIN inputs during the period from July 2010 to December 2012 (for which weekly CHLa measurements were available). Our results show that DIN inflow concentrations were a significant predictor of CHLa accounting for 58% of the variation (Figure 7). As with any correlation analyses, other factors, in this case temperature and water residence time, co-vary with our predictor variable.

However, these results, along with those from the bioassay experiments are consistent with the hypothesis that phytoplankton in the James respond to the concentration of nutrients in inflow which is determined by the balance between local point source inputs and watershed (riverine) runoff.

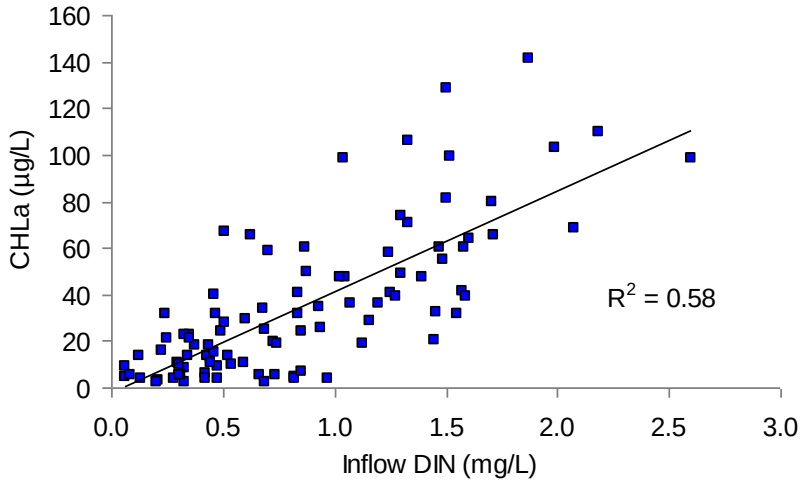


Figure 7. Inflow DIN concentrations as a predictor of CHLa in the tidal fresh James. Inflow concentrations take into account weekly input fluxes from riverine and point sources. CHLa data are measurements at JMS75 for July 2010 to December 2012.

We did not find evidence for spatial variability in the form or severity of resource limitation based on comparison of near-shore and main channel sites (H2). Prior monitoring had revealed differences in DIN concentrations between main channel and near-shore areas which were attributed to sediment denitrification. Results from bioassay experiments did not show differences in responses of main channel and near-shore phytoplankton to N addition and suggest that DIN gradients were too small, or too ephemeral, to affect phytoplankton N limitation. This segment of the James is well-mixed owing to the large tidal prism (relative to total volume); timescales of phytoplankton transport are likely too rapid to allow spatial differentiations in resource limitation. This finding has potential implications for modeling efforts as it excludes the need to separately parameterize resource-growth functions for main channel and shallow areas.

The hypothesis that phytoplankton in the tidal fresh James were principally limited by the availability of N (H3) was partially supported by the results of this study in that we observed responses to addition of N alone in 2 of 6 months. We did not find support for the hypothesis of preferential use among various forms of N (NH_4, NO_3, Urea) that would result in variable biomass yield (H4). It is well established that NH_4 uptake is more energetically efficient than NO_3 uptake, and it is presumed that dissolved inorganic forms of N are more available than dissolved organic forms. However, all three forms of N produced similar growth responses during the one experiment (June) when N was clearly limiting. Our findings do not preclude the possibility of preferential utilization when all three forms are simultaneously available (as in the river). A recent mass balance analyses for the tidal fresh James showed that NH_4, was preferentially retained over NO_3 despite the fact that NO_3 inputs occurred at the top of the study reach and NH_4 entered near the bottom (Bukaveckas and Isenberg, in review). The mass balance analysis, coupled with the bioassay results, suggest that all three forms should be considered as part of the bioavailable pool supporting phytoplankton production. These findings have potential

applications for depicting phytoplankton growth as a function of resource availability in developing a deterministic CHLa model for the James.

Lastly, we characterized algal growth responses to nutrient amendments over a range of irradiances representative of in situ light conditions to test for effects on nutrient utilization and biomass yield (H5). We observed positive responses to nutrient amendments over the full range of light conditions used in the bioassay experiments. However, larger responses were observed at the higher light levels representative of the broader, shallow segment of the river (near Hopewell) in comparison to the lower light levels occurring in the constricted, deeper channel. These findings are consistent with the hypothesis that the location of persistent algal blooms in the tidal fresh James is due to favorable light conditions arising from changes in channel geomorphometry. Thus the occurrence of chronic algal blooms in the tidal fresh segment can be attributed in part to natural features of estuarine morphology, as well as to proximal point source inputs. Understanding of the causes of spatial and temporal dynamics in algal blooms provides a basis for inferring system responses to nutrient management scenarios. Elevated CHLa in the region near Hopewell would likely persist even with mitigation of nutrient inputs, however, the magnitude and duration of algal blooms would be expected to diminish with reduced nutrient loads.

Part 2: Top down Controls on Phytoplankton by Consumers

Introduction

Consumers have complex influences on phytoplankton abundance and community composition. Grazing can mitigate eutrophication effects by reducing phytoplankton biomass (Cohen et al. 1984, Cloern and Alpine 1991, Ibáñez et al. 2012), or exacerbate problems by increased nutrient cycling and favoring cyanobacteria through selective removal of their competitors (Schaus et al. 2002, Vanni et al. 2006, Friedland et al. 2011). Grazing rates are influenced by many factors including consumer abundance, food quantity and quality, and temperature. In estuaries, the capacity for grazers to reduce phytoplankton has been well studied for commercial species such as the American Oyster and Atlantic Menhaden, while others have received less attention (Gottlieb 1998, Cerco & Noel 2010). Most aquatic ecosystems have experienced significant changes in grazer communities due to invasive species and eutrophication. Historical declines in shellfish are of particular concern when considering nutrient mitigation strategies and forecasting recovery (zu Ermgassen et al. 2013). Identifying the important grazers in the tidal fresh James River will improve our understanding of phytoplankton dynamics including the factors which favor occurrence of harmful algal blooms.

Grazer communities in tidal freshwaters are diverse in terms of taxonomy, life history, habitat and feeding style; this diversity contributes to the complex flow of energy in food webs (Hoffman et al. 2008, Benke et al. 2011). Key grazers include benthic (aquatic insects and bivalves) and pelagic (zooplankton and fish) organisms that feed on suspended (phytoplankton) and sedimented (phytodetritus) particulate matter. In estuarine systems with tidal-driven re-suspension, benthic feeders may act to control CHLa in the water column by consuming phytodetritus. Factors which enhance the ability of a consumer to constrain phytoplankton abundance include the proportion of their diet which is composed of algae, the abundance of consumers and their feeding rate. Grazing rates vary seasonally due to changes in temperature as well as food quality and quantity. While considerable attention has been given to tracking changes in algal abundance in the James River, little is known about the fate of this material. A nutrient mass balance for the tidal fresh James indicated that combined watershed and point source nutrient inputs account for only 20 and 36% of algal N and P demand, suggesting high rates of internal cycling which may be consumer-driven (Bukaveckas and Isenberg, *in review*). With this study we aim to identify important grazers in this system and obtain data that may be used to assess their impact on phytoplankton abundance. In addition to assessing the impacts upon phytoplankton, this work supports efforts to track the fate of algal toxins (see Part 3).

To identify benthic species which are potentially important grazers in this system, we reviewed the Chesapeake Bay Program's benthic macroinvertebrate survey data (2001-2010) for the tidal fresh James River. These values were converted to production estimates by multiplying biomass by literature values for taxon-specific P/B (production/biomass) ratios (Figure 8). Results indicate that the most productive species is *Rangia cuneata,* the common wedge clam, representing 88% of the estimated benthic secondary production. Prior work has shown that *Rangia* has the potential to control phytoplankton populations in other estuarine systems due to high clearance rates (Wong et al. 2010). A recent modeling study indicated that incorporating grazing by *Rangia* can improve Chesapeake Bay chlorophyll models because the clams impose an appreciable loss rate on phytoplankton (Cerco and Noel, 2010). Their modeling study relied

on grazing rates of American Oysters for parameterization due to the lack of available data on *Rangia* grazing rates. In order to improve our understanding of these filter feeders, and to improve model depictions of their effects on CHLa, we undertook a study to measure *Rangia* grazing rates. The measured grazing rates were subsequently used in conjunction with estimates of their abundance (CBP data) to derive community filtration rates for the tidal freshwater James River. We hypothesized that grazing rates would increase with water temperature but that in late summer, the presence of cyanobacteria and Microcystin could have an inhibitory effect.

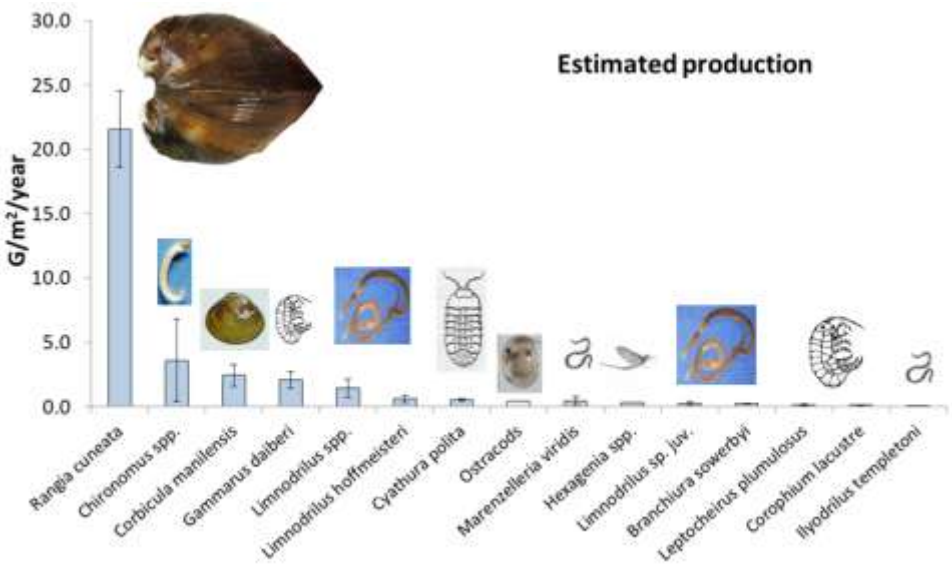


Figure 8. Benthic production (g/m2/year) in the tidal fresh James River derived from biomass (CBP benthic monitoring, 10 year average) and species-specific P/B values obtained from the literature.

Zooplankton grazing rates in the tidal fresh James were found to be low (<5% CHLa d^{-1}) in comparison to other estuaries (Bukaveckas et al. 2011). Therefore planktivorous fishes were the focus of efforts to assess pelagic grazing (Schaus et al. 2002, Lynch et al. 2010). The tidal fresh James River has large resident fish populations (e.g., gizzard and threadfin shad, blue catfish) as well as transient populations of Atlantic menhaden. Their feeding habits include pelagic filter-feeding (Atlantic menhaden, threadfin shad and young gizzard shad) and benthic detritivory (catfish, adult gizzard shad). Fish gut-content analysis has long been used to characterize diet; however most studies focus on identifiable remains from macrobiota. A large proportion of fish gut contents is un-identified amorphous material that arises from feeding on suspended and sedimented particulate matter. For example, analyses of James River catfish revealed that unidentifiable remains accounted for the largest proportion of gut contents in small size classes (<20 cm; VIMS Data Report Chesapeake Bay Trophic Interactions Laboratory). We measured CHLa concentrations in gut contents obtained from dominant fish species to assess their role as phytoplankton consumers. Data arising from this study can be used in conjunction with literature estimates of gut clearance rates to estimate CHLa removal rates by fishes.

Methods

Rangia Grazing Experiments

Rangia grazing rates were measured within 20 L mesocosms containing water obtained from the James River (near JMS75). Experimental design followed (Wong et al. 2010) with monthly trials performed during March to November 2012. Clams were obtained from a

location near JMS75 and kept overnight for acclimation to experimental temperature conditions. Half of the mesocosms were kept at ambient temperature in a Conviron growth chamber while the other half were kept in a room temperature (20^{o}C) water bath to assess the effects of temperature on grazing rates independent of changes in food quality and quantity. Six mesocosms were used for each temperature treatment (3 with, 3 without clams). Each mesocosm contained a similar mass of clams (3-10 individuals depending on size). The average body mass of clams (soft tissues) used in the experiments was 2.6 g ind^{-1} (range = 0.5 to 5.0 g ind^{-1}). Mesocosm were kept shaded in order to prevent phytoplankton growth and equipped with a circulating pump to maintain particulates in suspension. Dissolved oxygen was monitored during the experiment with a Hydrolab Sonde. Samples were taken at 0, 2, 4, 6, and 24 hours to measure TSS, CHLa and POC. CHLa, POC and TSS were measured ~weekly in the James to characterize seasonal variation in food resources. We derived clearance rates based on differences in concentration between control and experimental mesocosms. Slopes of these regression lines were used to determine clearance rates for CHLa, POC and TSS as mass/g clam dry weight/hour (Coughlan 1969).

Clearance rate (L/ g DW/ h) = [(slope((mg/L) / h))* mesocosm volume (L)) / clam dry mass](g DW) / (average concentration (mg/L))

Fish Grazing Estimates

We analyzed CHLa in gut contents of gizzard shad, threadfin shad, Atlantic menhaden and two size classes of juvenile blue catfish (<20 and 20-40 cm) Approximately 15-20 fish of each species were obtained monthly from the James River (near JMS75) via electroshocking (as available). Contents from the stomach were removed surgically for determination of wet weight. Organic matter and N content of gut materials were determined from CHN analysis of dried and ground samples. Samples for CHLa analyses were weighed and extracted in 90% buffered acetone prior to analysis on a TD-700 fluorometer. The dry weight of tissue and gut contents samples was determined after drying at 60^{o}C for 48-72 h. CHLa results are presented as µg CHLa/g dry weight of stomach contents. Because CHLa may have already degraded within the digestive tract of fish, these estimates are considered conservative.

Results

Rangia clearance rates were measured monthly from March to November to assess seasonal patterns arising from variation in water temperature and food conditions (Figure 9). Biomass-specific clearance rates were highest in Spring (March-May = 0.19 ± 0.03 L g^{-1} h^{-1}) and lowest in Summer (Jun-Sep = 0.07 ± 0.01 L g^{-1} h^{-1}). Clearance rates partially recovered in Fall, though values were still below those observed in Spring (Oct-Nov = 0.11 ± 0.03 L g^{-1} h^{-1}). Clams incubated at standardized (20^{o}C) and in situ (river) temperatures exhibited similar clearance rates. Statistically significant temperature effects on clearance rates were detected in only 1 of 9 experiments (August) when clearance rates at the standard temperature were lower than those at the ambient temperature. Low clearance rates in summer coincided with elevated Microcystin concentrations in the water column. Microcystin was a significant predictor of clearance rates with a non-linear model explaining 66% of the variation. The power model depicted a rapid decline in clearance at low Microcystin concentrations (<0.02 µg/L) with low and constant clearance rates at higher Microcystin levels.

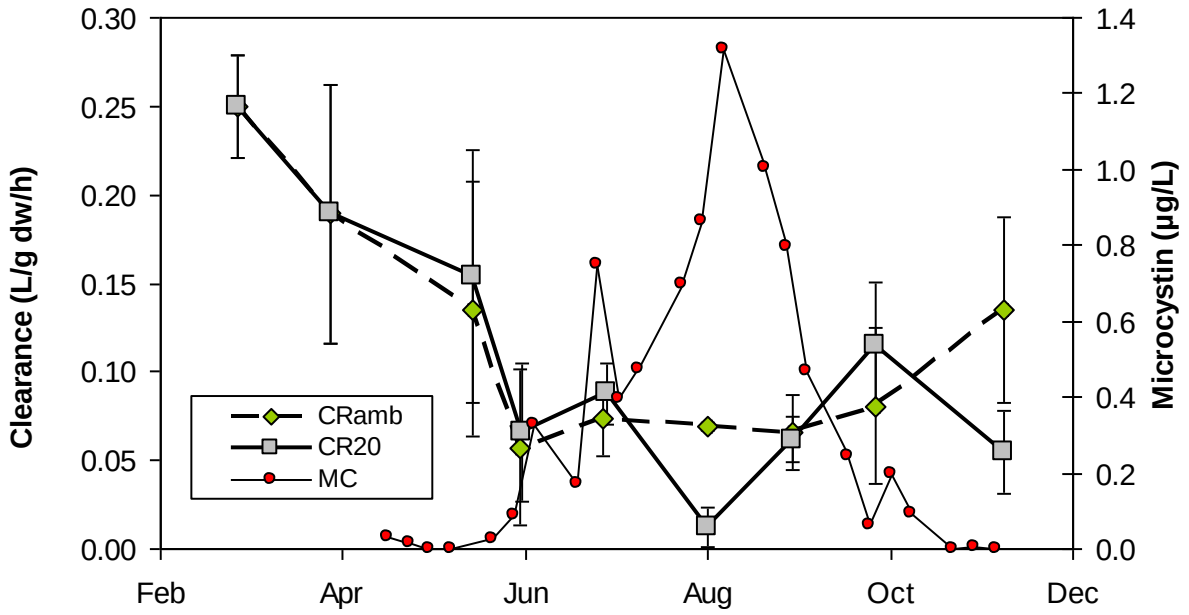


Figure 9. Biomass-specific clearance rates (L/g DW Clam/h) for the wedge clam *Rangia cuneata* and concentrations of the cyanotoxin Microcystin in the tidal fresh James River. Clearance rates were measured at ambient (in situ; CRamb)) and standard (20^{o}C; CR20) water temperatures.

Approximately 500 fish were collected for gut contents analyses during April to October. The number and types of fish were sufficient to derive 34 monthly, taxa-specific estimates of CHLa in gut contents (of possible 42 taxa-month combinations). We obtained at least 5 monthly estimates for 5 of the 6 taxa (excluding Atlantic menhaden; Figure 10). Within-species variability in CHLa ingestion was relatively low as standard errors of the monthly estimates averaged 22% of the mean. Highest CHLa was found in the stomach contents of pelagic fishes: young-of-the-year (YOY) gizzard shad = 224 ± 67 µg CHLa/g dm, threadfin shad = 136 ± 46 µg CHLa/g dm, and Atlantic menhaden = = 100 ± 49 µg CHLa/g dm (mean ± SE per unit mass of gut contents). CHLa concentrations in stomach contents of benthic detritivores were lower (33-49 µg CHLa/g dm for adult gizzard shad and two size classes of blue catfish). Highest CHLa ingestion when normalized to individual fish body mass was also observed in pelagic fishes (YOY gizzard shad = 1.91 ± 0.47 µg CHLa/g dm, threadfin shad = 1.72 ± 0.62 µg CHLa/g dm) owing to the high CHLa concentration of their stomach contents and relatively small body size. Lowest mass-specific CHLa ingestion was observed among the two size classes of blue catfish (0.19 ± 0.05 µg CHLa/g dm fish and 0.13 ± 0.03 µg CHLa/g dm fish for <20 cm and 20-40 cm, respectively). Highest CHLa concentrations occurred in June and July (1.31 and 1.33 µg CHLa/g dm fish, respectively) with lower values in April-May (0.11 and 0.39 µg CHLa/g dm fish, respectively) and September-October (0.64 and 0.56 µg CHLa/g dm fish, respectively).

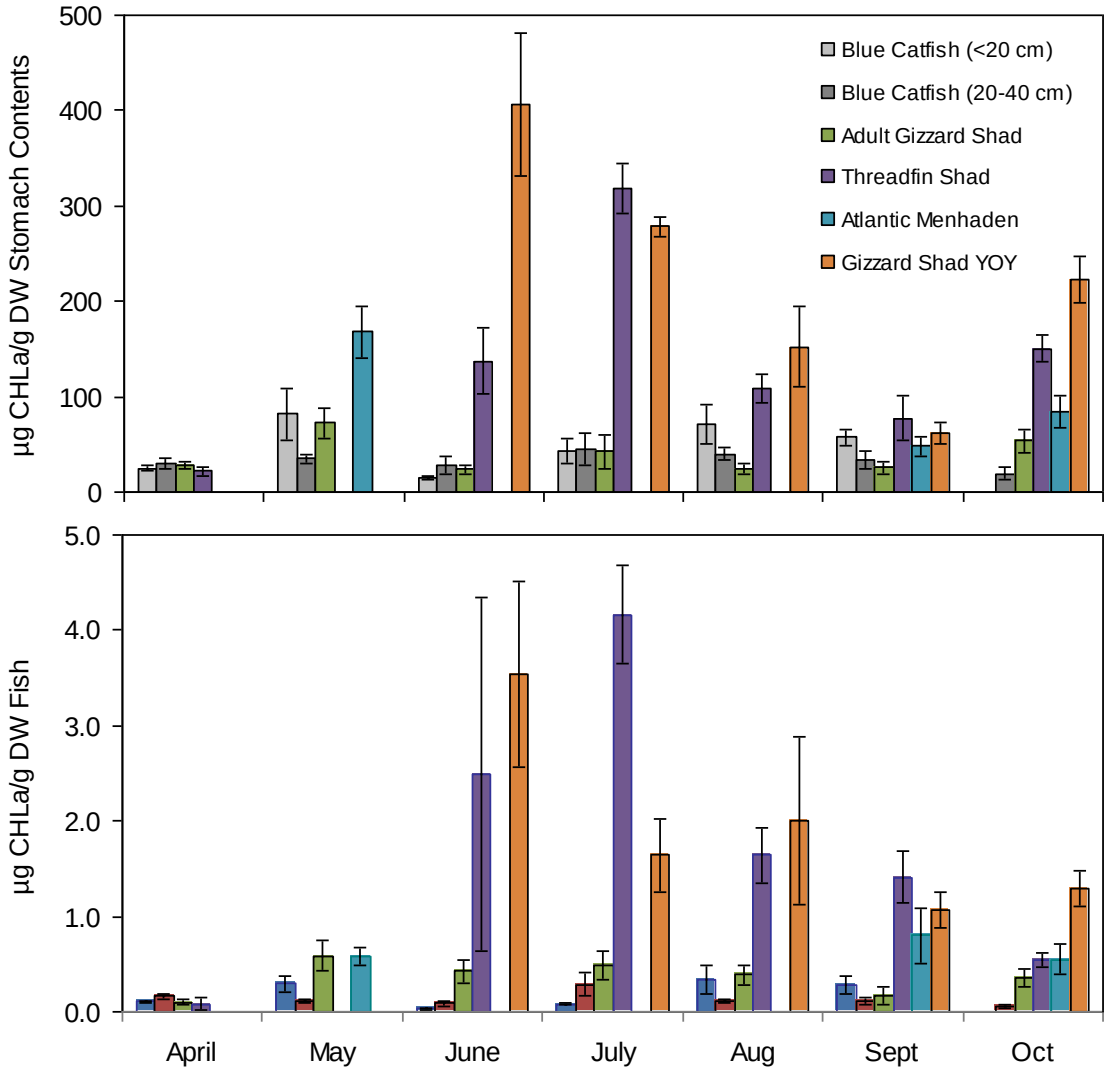


Figure 10. CHLa in gut contents of fishes from the tidal fresh James (collected near JMS75). CHLa normalized to total dry mass of gut contents (upper panel) and to individual fish mass (lower panel).

Discussion

Grazing rates for commercially-important bivalves such as oysters, mussels and edible clams are widely reported, whereas data for non-commercial species such as *Rangia* are sparse. Hartwell et al. (1991) measured clearance rates of *Rangia* from the Choptank River and reported mass-specific values of 0.38 to 0.72 L g^{-1} h^{-1} (mean ~0.5 L g^{-1} h^{-1}). Our measured clearance rates for the James were lower: range = 0.06 to 0.25 L g^{-1} h^{-1}, mean = 0.12 L g^{-1} h^{-1}. The *Rangia* used in the two studies were of similar size (mean = 2.6 and 2.2 g ind^{-1} for James and Choptank, respectively) and incubated at similar temperatures (Choptank mean = 21.1°C; James standard temperature = 20°C). Corresponding per capita rates (taking into account body size) were 26.4 L ind^{-1} d^{-1} (Hartwell et al. 1991) and 7.5 L ind^{-1} d^{-1} (this study). Holley and Foltz (1987) reported

rates of 16.4 L ind^{-1} d^{-1} for *Rangia* collected from a Louisiana bay. The two prior studies measured clearance rates on cultured algae whereas ours used naturally-occurring James River phytoplankton. Cultured phytoplankton used in the prior studies were selected for their edibility whereas native phytoplankton exhibit a diversity of size and nutritional properties which may account for the lower clearance rates observed in our study. Our comparison values were based on clearance rates of CHLa which were higher than those for POC or TSS, indicating selective feeding by *Rangia*.

Models depicting benthic filter-feeding effects on CHLa have relied on data from commercial species due to the lack of information on the non-commercial species which dominate tidal freshwaters (e.g., Gerritsen et al. 1994). The recent paper by Cerco and Noel (2010) used an oyster-based clearance rate of 20 L ind^{-1} d^{-1} to model grazing by *Rangia* and *Corbicula* in tidal fresh waters of Chesapeake Bay. Our highest measured rates (Spring) were appreciably lower (~12 L ind^{-1} d^{-1}) and these declined to just 4.1 L ind^{-1} d^{-1} during the period of summer algal blooms. Our findings suggest that models depicting benthic filter-feeder effects based on oyster grazing, or *Rangia* grazing on cultured algae, are likely to over-estimate their effects on CHLa in the tidal fresh James. Despite the lower values reported here, *Rangia* were found to exert an appreciable grazing effect in the James. Based on an average density of 30 ind m^{-2} (CBP monitoring, D. Dauer, ODU) and an average depth of 1.3 m in the region where algal blooms occur, we estimate that *Rangia* can remove up to 28% of CHLa per day. This estimate is based on maximal (Spring) filtration rates which declined to 10% CHLa d^{-1} during the period of summer algal blooms. Our estimates do not take into account re-cycling effects which can occur if vertical or lateral discontinuities in mixing result in re-filtering of water overlying clam beds (Cerco and Noel 2010). Recycling may be unlikely in the James given the strong tidal forcing. Incorporation of our Rangia clearance rates into the deterministic CHLa model being developed should allow for a more sophisticated approach to depicting benthic filter-feeding effects in the context of local hydrodynamics.

In addition to accurate estimates of clearance rates, realistic depiction of seasonal patterns in grazing is critical to modeling benthic filter-feeder effects on algal blooms. Models typically represent feeding activity as a positive function of temperature up to an optimal value (~20-25^{o}C), above which feeding rates plateau or decline (Gerritsen et al. 1994). This model assumes maximal feeding rates occur in summer, though our findings suggest that *Rangia* grazing in the James declines appreciably in summer to less than one-third of maximal (Spring) values. This decline was observed among *Rangia* incubated at both ambient (in situ) and standard (20^{o}C) temperatures. Viergutz et al. (2012) reported similar findings of large seasonal variation in clearance rates of *Corbicula* for the Rhine at both the ambient field temperature and a constant temperature (15^{o}C) which they attributed to "endogenous factors" such as spawning activity. We can not discount the possibility that spawning cycles and nutritional factors other than the presence of Microcystin may account for the summer decline in *Rangia* grazing observed in the James. For example, clearance rates are sensitive to particle concentrations, though we did not find a statistical relationship between filtration and TSS concentrations. Cyanobacteria are generally known to be a poor food source for consumers (Brett et al. 2009) and recent work has shown acute dietary effects of *Microcystis* on zooplankton for both toxic and non-toxic strains (Ger et al. 2010). Our findings suggest a potential negative feedback of harmful algal blooms via suppression of consumer grazing. This feedback would exacerbate cyanobacterial blooms by inhibiting grazing of one of the dominant consumers (by biomass and production) in the tidal fresh James and could lead to long-term declines in grazer abundance. Inhibition of summer

feeding by harmful algal blooms would result in a negative metabolic balance during summer when warmer temperatures result in higher respiration. Data from stable isotope analyses support this view. The carbon isotopic signature of *Rangia* more closely resembles that of seston collected during non-bloom periods, suggesting that inhibition of grazing minimizes the contribution of summer algal blooms to *Rangia* production. Further work is needed to validate Microcystin effects on living resources, and for *Rangia* specifically, given its potential importance in grazing on phytoplankton.

Analysis of fish gut contents revealed measurable CHLa concentrations in all species and months. Within-month, intra-specific variation was low whereas inter-specific differences were large. Small, pelagic fishes (YOY gizzard shad, threadfin shad and Atlantic menhaden) consumed 7 times as much CHLa on a per body mass basis in comparison to benthic detritivores (adult gizzard shad and blue catfish). The role of planktivorous fish in trophic dynamics is well-studied in lakes due to their effects on the size structure of zooplankton communities, and their role in trophic cascades, whereby increases in planktivores suppress zooplankton and release phytoplankton from grazer regulation (Brooks and Dodson 1963, Carpenter et al. 1986). Less is known regarding their role in estuarine food webs where studies have largely focused on commercially important species such as Atlantic menhaden. Atlantic menhaden are obligate filter-feeders which passively ingest a mixture of phytoplankton, zooplankton and detritus in proportion to their ambient concentrations. Menhaden can locally deplete phytoplankton densities (Oviatt et al. 1972) and have the capacity to affect top- down control on plankton communities in Chesapeake Bay due to their high filtration capacities (Gottlieb 1998). To assess their importance in the tidal fresh James, we derived an average clearance rate based on average per capita CHLa in gut contents (26 µg CHLa ind^{-1}), average CHLa in the water column (28.7 µg CHLa L^{-1} at sites JMS56, 69 and 75) and assuming a gut turnover time of 2 h (Gottlieb 1998, Friedland et al. 2005). The average per capita clearance rate was 11 L ind^{-1} d^{-1} which is comparable to the value reported by Lynch et al. (2010; 14 L ind^{-1} d^{-1}) for Chesapeake Bay menhaden feeding at CHLa concentrations similar to those observed in the tidal fresh James during our study period. Clearance rates based on CHLa are likely to be conservative as CHLa is known to degrade during passage from the fore-gut to hind-gut (Friedland et al. 2005). Therefore we also derived estimates based on the total dry mass of gut contents and concentrations of total suspended solids in the water column. TSS-based clearance rates were an order of magnitude higher (176 L ind^{-1} d^{-1}) and corresponded to ingestion rates equivalent to ~21% of biomass per day.

In this preliminary work we have established the potential importance of fish grazing as a "top-down" control on CHLa in the tidal fresh James. Clearance values for pelagic species compared favorably to those derived for benthic filter-feeders. In considering their relative importance to controlling algal blooms, it should be noted that CHLa in fish gut contents was maximal in June-July, whereas *Rangia* grazing declined in summer. Our findings to date consider only the contribution of CHLa to fish diets, and not fish density. A critical need for evaluating fish grazing effects on CHLa is data characterizing the abundance of dominant fish species as these data would allow us to incorporate fish grazing effects in modeling CHLa. CHLa data on gut contents were also found to be useful for interpreting inter-specific variation in Microcystin contamination of fish tissues. These relationships are described in the following section (Part 3).

Part 3: Microcystin in the James River Food Web

Introduction

Harmful Algae Blooms (HABs) are a growing worldwide concern particularly in coastal areas with large anthropogenic nutrient loads. Harmful algae produce secondary metabolites which act as toxins and therefore pose threats to human health and living resources (De Figueiredo et al. 2004). Microcystin, a hepatotoxin, has received considerable attention recently due to cancer promoting properties and ubiquitous presence in freshwaters (Chorus and Bartram 1999). Microcystin exposure can elicit a range of physiological responses including increased heart rate, osmoregulatory imbalance, reduction of antioxidant formation, loss of liver function and mortality (Best et al. 2001, Bláha et al. 2004, Malbrouck & Kestemont 2006, Prieto et al. 2007, Ibelings & Havens 2008).

Microcystin has been found in a diverse group of organisms including fish, insects, crustaceans, bivalves, amphibians, birds and mammals (Wilson et al. 2008, Garcia et al. 2010, Papadimitriou et al. 2010, Poste et al. 2011, Acuna et al. 2012). Exposure is thought to occur primarily through dietary consumption though little is known about the factors which contribute to variable exposure and toxin contamination among consumers (Kozlowsky-Suzuki 2012). Human exposure to Microcystin occurs through drinking water, recreational contact or fish consumption. Microcystin is water stable and resistant to boiling thus posing a threat to drinking water supplies and fish consumption. The World Health Organization (WHO) has issued guidelines of 1 µg L^{-1} for drinking water, 5 µg L^{-1} for recreational contact and 0.04 µg kg^{-1} body weight d^{-1} for consumption. Microcystin concentrations are typically highest in liver and viscera (Wilson et al. 2008, Garcia et al. 2010, Papadimitriou et al. 2010); shellfish may therefore pose a greater threat for human exposure because consumable portions include non-muscle tissues. Human exposure to Microcystin raises concerns regarding impairment of designated uses such as swimability and fishability and therefore requires an assessment of the magnitude and duration of HAB events and toxin propagation in food webs.

While little is known about the factors that regulate Microcystin production, blooms have been observed more frequently in warm, shallow, CHLa-rich waters that receive large anthropogenic nutrient loads. (Moisander et al. 2009, Poste et al. 2011). The tidal fresh segment of the James River Estuary shares a number of features in common with these systems including anthropogenic nutrient inputs, shallow depths, high CHLa and high cyanobacteria abundance (Marshall et al. 2008, Bukaveckas et al. 2011, Bukaveckas and Isenberg *in review*). Cyanobacteria abundance is a useful indicator of the potential for a Microcystin event but not all cyanobacteria are capable of producing the toxin, and those which are capable vary in their level of toxin production (DeMmott and Moxter 1991). Assessment of the occurrence of Microcystin events has been aided by advances in the measurement of Microcystin using Enzyme Linked ImmunoSorbent Assays (ELISAs). As part of a broader effort to characterize harmful algal blooms in the James, we undertook a study to monitor the occurrence of Microcystin in water, sediments and biota of the tidal freshwater segment. Results from this study provide the first comprehensive assessment of Microcystin in the James and a basis for determining whether existing CHLa standards are suitable to protect living resources from HAB effects (Tango and Butler 2008, Davis et al. 2010, Davis and Gobler 2011).

Methods

Sample Collection

We collected samples of water, sediments and tissues to characterize the presence of Microcystin in the tidal fresh James River during May-November 2012. Tissue samples included muscle and liver/viscera from bivalves (*Rangia*), blue crabs, gizzard shad, threadfin shad, Atlantic menhaden and blue catfish. Water samples were collected ~weekly at 3 main channel sites (JMS 69, 75, 99; stations are CBP long-term monitoring sites designated by distance from CB) and one off-channel site located in the Appomattox River sub-estuary (APP1.53). Samples from these 4 sites were collected on 17 dates in conjunction with weekly monitoring activities conducted by VCU for the City of Richmond and this project (N=68). Ancillary data included determinations of CHLa, nutrients and phytoplankton counts (samples provided to H. Marshall, ODU). CHLa, Microcystin and phytoplankton data were also available for 6 monthly samples collected at the Rice Pier in conjunction with bioassay experiments. Thus the pooled dataset for analyzing CHLa-Microcystin-cyanobacteria relationships consisted of 74 observations. Sediment samples were collected monthly at 3 sites (Rice Pier, Tar Bay, and Turkey Island). The Rice Pier and Tar Bay sites are located in the region of persistent algal blooms (near JMS75); Turkey Island is located in the upper, constricted segment of the tidal freshwater zone where CHLa and Microcystin concentrations are typically low (Bukaveckas et al. 2011). *Rangia* were obtained monthly in conjunction with grazing experiments (see Part 2) at a site located near JMS75. Clams were held in particle-free water for 48 hours to allow for gut clearance and prevent Microcystin contamination from consumed material. Blue crabs were collected monthly from crab pots deployed near the Rice Pier. Fish samples were obtained in conjunction with gut contents analyses (see Part 2). Ten individuals for each target species (as available) were collected each month. Univariate, least squares regressions were used to test for relationships between CHLa and Microcystin, and between CHLa in fish gut contents and Microcystin in liver tissues.

Microcystin Extractions

Water samples were thawed and refrozen 2 times prior to analysis in order to release Microcystin from cells (as per manufacturer protocols). In order to improve extraction efficiency, water samples were also microwaved and sonicated (Silva-Stenico et al. 2009). Samples were analyzed using a high sensitivity Microcystin ELISA Kit (Abraxis) with an ELISA plate reader. To extract Microcystin from sediment and tissues we used methods described by Wilson et al. (2008) and Garcia et al. (2010). Samples (tissue or sediment) were dried at 60^{o}C for 48 hours, ground with a mortar and pestle and extracted in 75% aqueous methanol for 24 hours. Extracts were then centrifuged and supernatant collected. Prior to ELISA analyses subsamples were diluted with DI such that sample to be run on the ELISA plate was no greater than 5% methanol. Samples were analyzed using a Microcystin ELISA kit (as above) and expressed as mass/dry weight. To determine the efficiency of extraction techniques, samples were spiked with a known quantity of Microcystin. Percent recovery for various types of samples (Table 5) was within the range of previously published values (50% to 115%; Cong et al. 2006, Wilson et al. 2008, Garcia et al. 2010).

Material	Recovery (%)	Source
Water	94 ± 6%	This study
Sediment	90 ± 11%	This study
water/seston	91 - 106%	Cong et al. 2006
	92 - 111%	Wang et al. 2007
	92%	Wilson et al. 2008
Blue Crab Muscle	104 ± 7%	This study
	50%	Garcia et al. 2010
Blue Crab Viscera	67 ± 7%	This study
	98%	Garcia et al. 2010
Fish Liver	74 ± 15%	This study
Fish Muscle	89 ± 12%	This study
Fish Tissues	65%	Wilson et al. 2008
	68%	Ernst et al. 2005
	68%	Ibelings et al. 2005
Rangia Muscle	84 ± 9%	This study
Rangia Viscera	64 ± 1%	This study

Table 5. Recovery of Microcystin from spiked samples in this and previously published studies

Results

Microcystin was detected in 104 of 105 water samples collected at five stations (JMS56, 69, 75, 99 and APP1.3) between May 8 and November 1, 2012. Highest concentrations were measured at JMS69 (Jun-Sep mean = 0.59 ± 0.09 µg L^{-1}); high values were also observed upriver at JMS75 (mean = 0.42 ± 0.06 µg L^{-1}) and downriver at JMS56 (mean = 0.37 ± 0.08 µg L^{-1}; Figure 10). Lowest concentrations were measured at the most upriver site (JMS99; mean = 0.06 ± 0.01 µg L^{-1}) where concentrations never exceeded 0.15 µg L^{-1}. Two seasonal peaks in Microcystin occurred on July 17 (mean = 0.92 µg L^{-1} for JMS56, 69 and 75) August 28 (mean = 0.78 µg L^{-1}). By November 27, Microcystin was undetectable at all stations. CHLa was found to be a significant predictor of weekly variation in average Microcystin concentrations among sites located in the region of persistent algal blooms (JMS56, 69 and 75; $R^2 = 0.54$, $p < 0.002$; Figure 11). Total phytoplankton, cyanobacteria and *Microcystis* cell densities and biomass were all found to be significant predictors of variation in Microcystin ($p < 0.01$, $N = 74$). The proportion of variation explained by linear, univariate models was relatively low ($R^2 = 0.08$ to 0.32) but graphical analyses of these relationships revealed a strong threshold effect (Figure 11). At cyanobacteria cell densities below 10,000 cells ml^{-1}, Microcystin concentrations were uniformly low (< 0.02 µg L^{-1}). At higher cell densities, Microcystin concentrations were higher (0.02 – 1.2 µg L^{-1}).

Microcystin was detected in 11 of 60 sediment samples collected from three stations during May to October. At the two sediment sampling sites located in the region of high CHLa (Rice Pier and Tar Bay), Microcystin was consistently present in the sediments during July, August and September (Figure 12). Highest Microcystin was measured at Tar Bay in September, which coincided with highest sediment CHLa concentrations. At the upriver reference site (Turkey Island), Microcystin was measurable only in September. Microcystin concentrations in sediment (mean = 0.0004 µg g^{-1} DM; max = 0.0026 µg g^{-1} DM) were four orders of magnitude lower than those measured in the water column when the latter are expressed per unit dry mass (based on TSS, range = 15-30 µg MC g^{-1} DM).

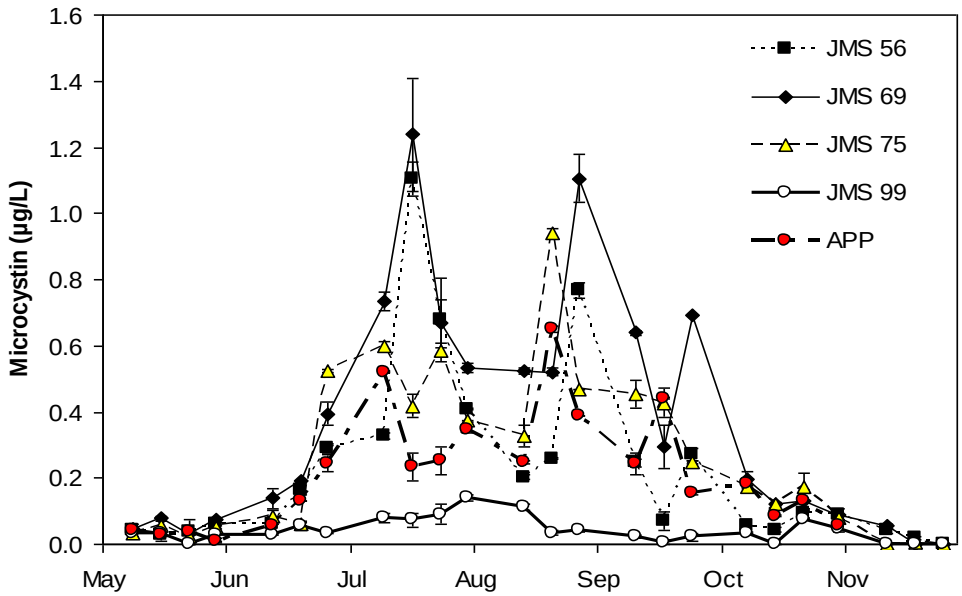


Figure 10. Water column Microcystin concentrations at 4 stations located in the main stem of the tidal fresh James River (JMS56-99; values denote distance in river miles from CB) and one off-channel site located in the Appomattox sub-estuary (APP).

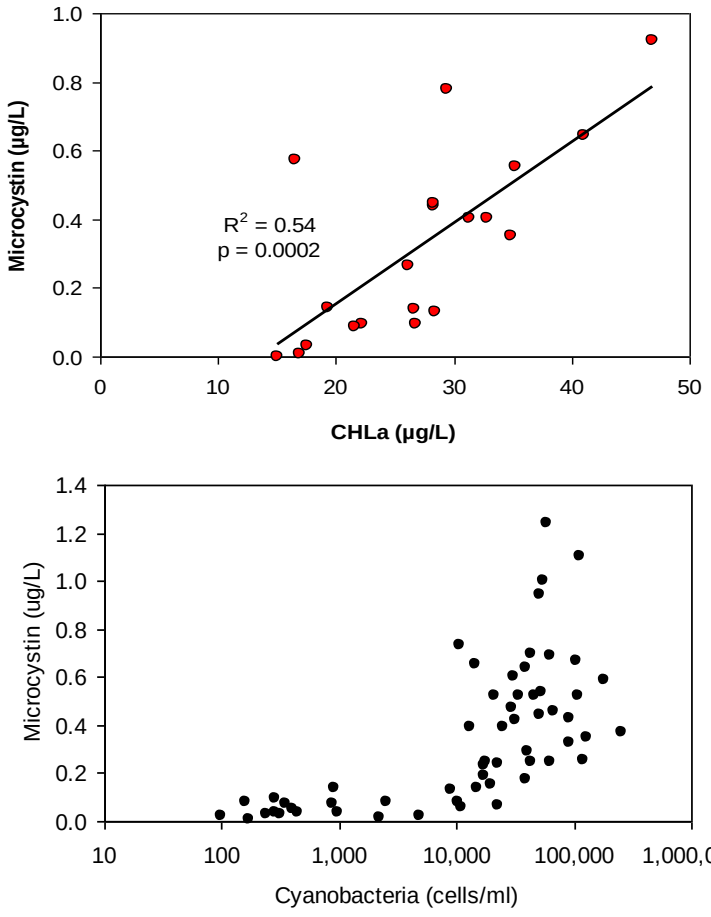


Figure 11. (Top) CHLa as a predictor of weekly variation in Microcystin. Data are average values for JMS75, 69 and 56 during June-November 2012. (Bottom) Relationship of Microcystin concentrations to cyanobacteria cell densities based on weekly samples from JMS99, 75, 69 and APP1.3 during May-September 2012.

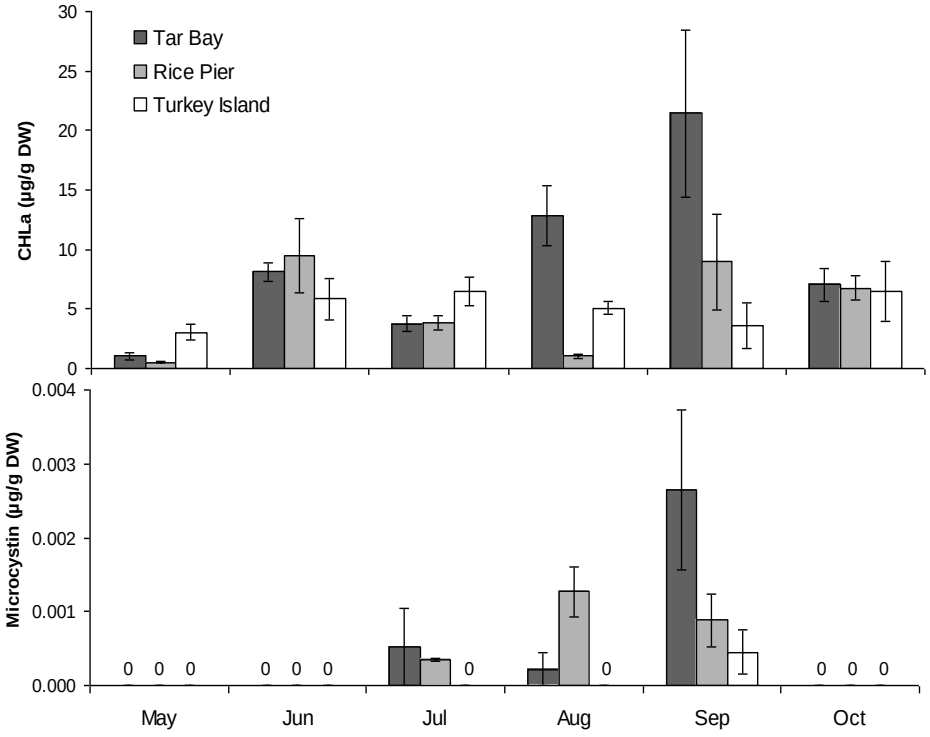


Figure 12. Sediment Microcystin and CHLa concentrations (mean ± SE) at 3 stations located in the tidal fresh James River. Two sites (Tar Bay, Rice Pier) were located in the region of high water column CHLa (near JMS75) and one site (Turkey Island) was located upriver in the low CHLa region.

Microcystin was detected in liver/viscera tissues of 67% of individuals collected during May-October 2012 (inclusive of fish and shellfish). Highest incidence of toxin contamination in liver/viscera was observed in August (94%) and September (83%; Figure 13). Proportion detection generally increased from May through September coincident with rising levels in the water column. The proportion of individuals with measureable toxin levels in muscle tissue was lower (mean = 14% for all species and months). Muscle tissues also exhibited consistently lower concentrations of Microcystin in comparison to liver/viscera. Highest incidence of Microcystin contamination occurred in blue crabs (viscera = 100%; muscle = 64%). Occurrence of contamination among other taxa ranged from 40 to 80% (liver/viscera) and from 0 to 22% (muscle). Microcystin accumulation in fish was higher among planktivores (Threadfin Shad, YOY Gizzard Shad, Atlantic Menhaden) in comparison to benthic detritivores (Blue Catfish, Adult Gizzard Shad; Figure 14). These findings are consistent with observed differences in Microcystin and CHLa concentrations between suspended and sedimented materials. CHLa concentrations in fish gut contents were found to be a significant predictor of inter-specific differences in liver Microcystin concentrations. To our knowledge, this is the first study linking consumer feeding habits with Microcystin exposure.

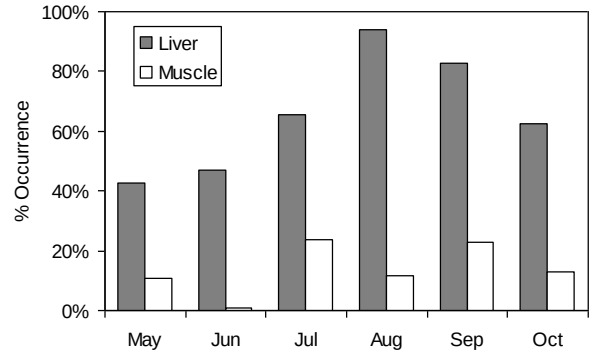


Figure 13. Proportion of individuals among fish and shellfish species exhibiting the presence of Microcystin in liver (or viscera) and muscle tissues.

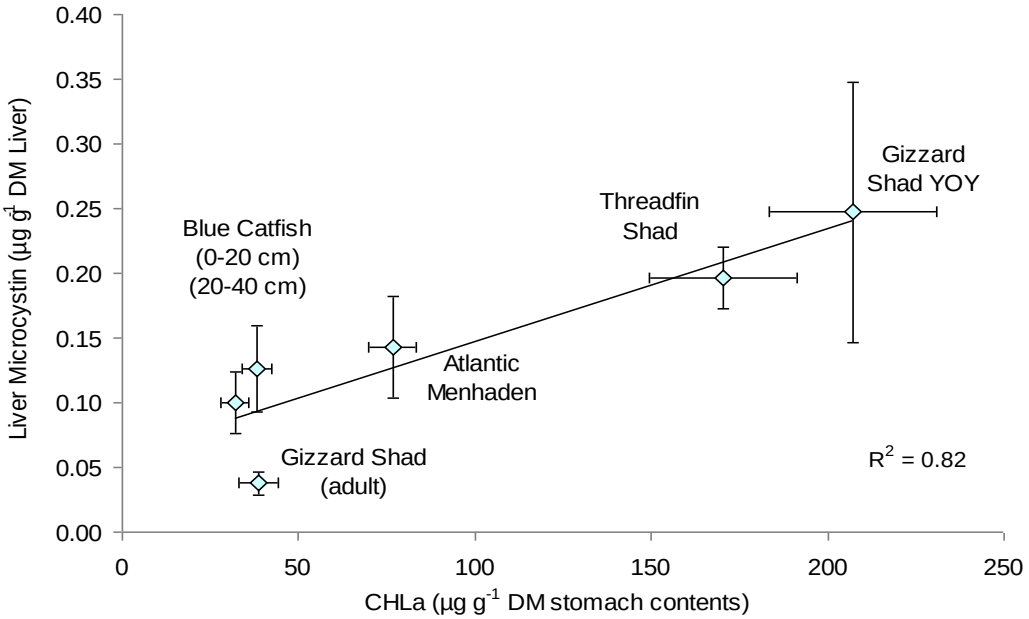


Figure 14. CHLa concentration in stomach contents predicts liver Microcystin concentrations in fish species of the tidal fresh James (collected near JMS75).

Discussion

This study is the first regular monitoring of Microcystin in the tidal fresh James and also reports the first measurements for the presence of toxins in living resources. Microcystin was consistently found in the water column (99% of samples tested). The toxin was already present in May when sampling was initiated and persisted through late November. Concentrations in the James exceeded the 1 µg L^{-1} drinking water standard (WHO) on two dates (July 17 and August 28) at stations JMS56 and JMS69. None of the samples collected at APP1.53 (a designated public water supply) exceeded the WHO drinking water guideline. No samples collected at any site exceeded the contact (recreational) standard of 5 µg L^{-1}. Peak levels were observed in the segment of the James where persistent, elevated CHLa concentrations occur (stations JMS75-56). CHLa was found to be a significant predictor of Microcystin which accounted for over 50% of the variation in averaged values for the three stations located in this region (JMS75, 69 and 56). Phytoplankton metrics, such as cyanobacteria and *Microcystis* cell densities, were also found to be significant predictors of variation in Microcystin, though the predictive power of

these relationships was weaker than that of CHLa (R^2 ~ 0.25). Although the predictive power of the various linear and non-linear models that were tested was low, there was a visually apparent threshold (~10,000 cyanobacteria cells/ml) below which Microcystin concentrations were uniformly low (<0.2 µg/L). Overall, results from the 2012 monitoring suggest that output from a deterministic CHLa model could be used to predict the occurrence and magnitude of cyanotoxin events in the James. Further study is required to assess inter-annual variation in the CHLa-Microcystin and Microcystin-cyanobacterial relationships. Low Microcystin concentrations at JMS99 indicate low potential for HAB effects on living resources in the upper, constricted segment of the tidal fresh James. We are unable to establish a downstream limit for HAB effects as toxin concentrations at our most seaward station (JMS56) were comparable (within ~30%) of peak values at JMS69. Prior work has shown that the toxin can be transported from freshwaters with toxic effects on marine species (Miller et al. 2010).

Microcystin concentrations in the James were similar to those reported in other systems where elevated tissue concentrations were observed in fish and macroinvertebrates (e.g., Wilson et al. 2008; Garcia et al. 2010). We found widespread occurrence of toxin contamination in all species of fish and shellfish sampled from the James. Peak occurrence of toxin contamination (~80% of individuals) was observed in months with highest Microcystin in the water column (July-September). Microcystin was measurable in liver tissues even in May (e.g., 71% of 20-40 cm blue catfish and 100% of blue crabs) The toxin is thought to be metabolized in the body thus limiting potential health effects to periods when toxin-producing blooms occur (Dionisio Pires et al. 2004). Our findings suggest that either tissue concentrations are sensitive to very low levels of Microcystin in the water column (mean = 0.03 µg L^{-1} during May 8-22), or that the toxin persist in tissues well-beyond bloom periods. Ozawa et al. (2003) reported measurable levels of Microcystin in freshwater snails during the Fall and Winter following a spring cyanobacterial bloom. These findings suggest that health effects associated with the toxin may be occur outside of bloom periods when the toxin is produced. Sampling of living resources during the spring, pre-bloom period would allow us to determine whether tissue concentrations re-set over winter, or whether toxins, and potential health effects, persist year-round.

Average liver toxin concentrations varied 10-fold among species (e.g., *Rangia* = 0.023 µg g^{-1} DM; Gizzard shad YOY = 0.323 µg g^{-1} DM); this range of variation was small in comparison to previously published fish and shellfish values which varied by four orders of magnitude (Figure 15). As has been reported in other studies, we observed lower toxin levels in muscle tissues relative to liver and viscera (Papadimtriou et al. 2008; Wilson et al. 2008; Garcia et al. 2010). This has positive implications for human exposure, though it should be noted that consumption of blue crabs may include non-muscle tissues. Also, apex predators such as osprey and bald eagles are known to consume non-muscle tissues. In the James, blue crabs exhibited the highest occurrence of muscle contamination (64% of individuals) and the highest concentration of toxin in muscle tissue. Our estimates of Microcystin concentrations in blue crab muscle tissue (0.018 µg g^{-1} DM) were similar to those previously reported by Garcia et al (2010) for a eutrophic Louisiana estuary (0.021 µg g^{-1} DM). Laboratory studies by Dewes et al. (2006) on an estuarine burrowing crab (*Chasmagnathus*) demonstrated that tissue concentrations of Microcystin exceeding 0.013 µg g^{-1} induced physiological and biochemical imbalances. We observed 10-fold higher concentrations in the James (viscera = 0.118 µg g^{-1}) suggesting that cyanobacteria blooms may adversely affect blue crab populations. To assess implications for human health, we compared Microcystin concentrations in blue crab muscle to WHO tolerable daily intake (TDI) guidelines for human consumption (0.04 µg kg^{-1} body mass d^{-1}). Following

calculations of Garcia et al. (2010; portion size = 300 g WM; body size = 60 kg), we found that monthly averages of Microcystin levels in blue crabs ranged from 31% (July) to 150% (August) of TDI (2012 mean = 73%). Eleven of 65 blue crabs collected for Microcystin analyses exhibited muscle Microcystin concentrations above the TDI guidelines, though it is important to note that chronic exposure at low concentrations may also cause health effects.

An important and novel finding from this study is that dietary exposure to Microcystin within food webs can be linked to feeding habits. We found that pelagic-feeding, planktivorous fishes had higher levels of CHLa in their gut contents as well as higher concentrations of Microcystin in their tissues compared to benthic-feeding detritivores. This finding suggests that at-risk species for toxic effects include Atlantic menhaden, YOY gizzard shad, threadfin shad and anadromous shad species. Benthic feeders such as juvenile catfish and adult gizzard shad experience lower Microcystin exposure. These differences reflect the orders of magnitude lower concentrations of Microcystin in sediments in comparison to suspended particulate matter. Lower sediment concentrations are likely due to dilution of phytodetritus settling from the water column by the much larger pool of sedimented material as well as post-depositional biodegradation of Microcystin (Grützmacher et al. 2010). The ratio of CHLa to Microcystin in suspended particulate matter was ~80:1, whereas the same ratio for surficial sediments was >1,000, suggesting that Microcystin has a substantially higher degradation rate. It has also been reported that Microcystin can adsorb to clay particles and thereby become resistant to conventional extraction procedures. Rinta-Kanto et al. (2009) attributed the lack of Microcystin in Lake Erie sediments to this mechanism, though other studies have reported high values in sediments using similar extraction techniques (e.g., 0.168 µg g^{-1} DM in Lake Taihu, China, Chen et al. 2008; 0.38 µg g^{-1} DM in Brno Reservoir, Czech Republic, Babica et al. 2006). Our sampling of the food web focused on primary and secondary consumers as Microcystin is not thought to bioaccumulate (Kozlowsky-Suzukie et al. 2012). Data on tertiary consumers in the James is limited to two taxa (blue crabs and blue catfish >40 cm) which show contrasting results. Blue crab tissue concentrations were high in comparison to known prey such as *Rangia*, whereas large catfish exhibited lower Microcystin concentrations (0.026 µg g^{-1} DM) relative to smaller, detritivorous catfish (<20 cm = 0.086 µg g^{-1} DM; 20-40 cm = 0.093 µg g^{-1} DM).

In summary, this comprehensive assessment of Microcystin in the tidal fresh James revealed widespread occurrence of the toxin in water, sediments and living resources. The presence of the toxin was directly related to the magnitude and duration of algal blooms as evidenced by elevated Microcystin concentrations in the region of JMS56-75 during July-September. Toxin contamination of fish and shellfish tissues followed seasonal patterns in water column CHLa, cyanobacteria and Microcystin. The overlapping distribution of blue crabs and *Rangia* in the tidal fresh James directly links cyanobacteria in the water column to human exposure via benthic filter-feeders and their predators. Further work to be proposed will include a spatial component for characterizing toxin levels in water and living resources to assess the longitudinal scale of toxin transport and exposure.

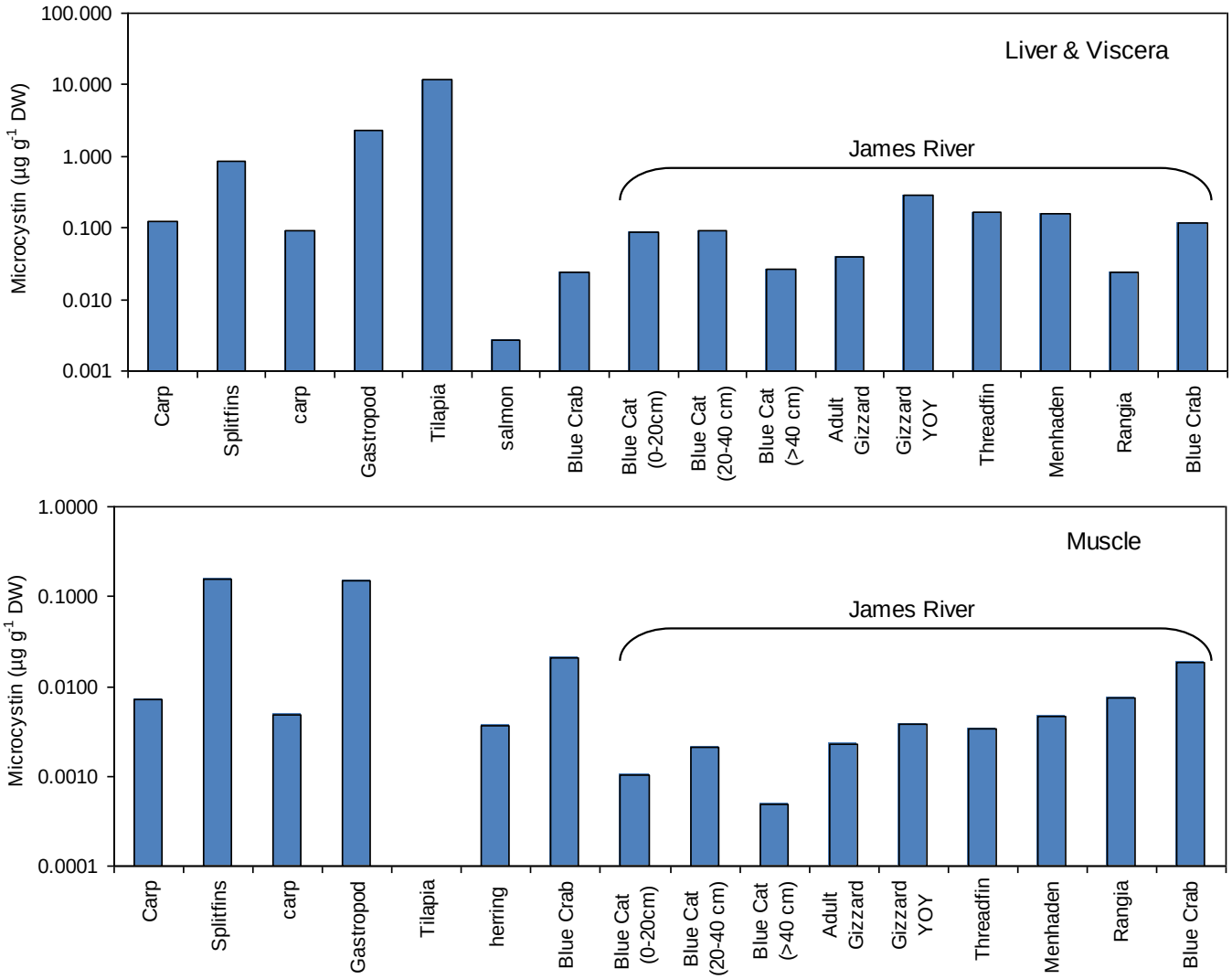


Figure 15. Comparison of Microcystin concentrations in liver/viscera and muscle tissues of species occurring in the tidal fresh James River with previously published values from Zhang et al. (2007), Deblois et al. (2008), Papadimtriou et al. (2008), Garcia et al. (2010) and Berry et al. (2011).

Appendix

In addition to the primary research questions addressed in Parts 1-3, we present here additional data and analyses based on our weekly monitoring program initiated in July 2010. The 2.5-y time series allows us to evaluate some aspects of the 2012 results (e.g., CHLa) from a multi-year perspective. Data from JMS75 show well-defined bloom periods in each year corresponding to seasonal patterns in water temperature and residence time (Figure A1). The periods of elevated CHLa (>20 µg L^{-1}) were associated with water temperature >15^{o}C, water residence >10 d, elevated TP (>0.80 mg L^{-1}) and depleted DIN (<0.20 mg L^{-1}). Intervening periods (~November-April) were characterized by low CHLa (<10 µg L^{-1}), low water temperature, short water residence time, elevated DIN and low TP. Seasonal patterns of TP showed good correspondence with CHLa, whereas TN did not. The positive association between TP and CHLa suggests that during summer, low-discharge conditions, the bulk of TP is in algal biomass. In contrast, algal-N likely accounts for a small fraction of PON and TN.

We compared the magnitude, duration and frequency of algal blooms in 2011 and 2012, the two years for which we had year-round monitoring (Table A1). Annual average CHLa values were similar in 2011 (25.6 ± 3.6 µg L^{-1}) and 2012 (24.1 ± 2.5 µg L^{-1}) though peak values were greater in 2011 than 2012 (79.7 and 66.7 µg L^{-1}, respectively). In both years, approximately half of the collections yielded CHLa values <20 µg L^{-1}; thus, the number of days when CHLa exceeded this value was ~178 in each year. The principal difference between the two years was that CHLa attained higher concentrations in 2011 (e.g., mean = 59.6 µg L^{-1} during June-August) but values were generally below 20 µg L^{-1} thereafter. In 2012, average concentrations for the corresponding time period were lower (36.3 µg L^{-1}) but values exceeding 20 µg L^{-1} persisted through September-November. These differences in Fall CHLa correspond to differences in water residence time as the absence of high discharge events in Fall 2012 resulted in longer residence time (mean = 15 d) in comparison to the same period in 2011 (mean = 10 d).

Restricting our analyses of inter-annual variation in algal blooms to a July-August time frame allowed us to include results from 2010. Inter-annual variation in July-August average CHLa was large (~2-fold) with a declining trend observed from 2010 (102.8 µg L^{-1}), to 2011 (64.0 µg L^{-1}), and 2012 (35.8 µg L^{-1}). POC concentrations (CV = 12%) and TP (CV = 6%) were more similar across years. Furthermore, assessments of the severity of algal blooms based on these three metrics yield different outcomes as POC (3.00 mg L^{-1}) and TP (0.104 mg L^{-1}) were lower in 2010 when compared to 2011 (POC = 3.82 mg L^{-1}; TP = 0.116 mg L^{-1}) and 2012 (POC = 3.41 mg L^{-1}; TP = 0.117 mg L^{-1}). These findings point out the challenges to assessing trophic state of the James based on CHLa alone.

Variable ratios of C:CHLa and CHLa:TP complicate the use of algal C-based growth models in predicting CHLa and the response to changing nutrient loads. To evaluate this issue, we derived C:CHLa ratios from our weekly data (2011 and 2012) as well as from the long-term DEQ data (1994-2011). We used data collected from April through October to minimize the influence of high discharge events which deliver terrestrial POM to the James. Although the VCU and DEQ monitoring programs share a number of sampling locations, comparisons of these data are complicated by differences in methodology for the analysis of CHLa and particulate carbon. DEQ reports phaeophytin-corrected CHLa concentrations whereas VCU measures total

CHLa. DEQ measures total particulate C (inorganic and organic fractions) whereas VCU measures particulate organic carbon. Despite these differences, VCU data fall along expected values based on the long-term DEQ monitoring (Figure A2). This finding suggests that methodological effects are small in comparison to inter-annual variability in PC and CHLa. Within a given year, there was a strong correlation between these parameters (R^2 = 0.78 to 0.93) as shown by results from the last two years for both monitoring programs (Figure A3). C:CHLa ratios obtained in the same year (2011) were similar in the weekly VCU dataset (46 µg:µg) and the monthly DEQ dataset (56). Pooling all DEQ data into a single regression yielded a C:CHLa of 47. We have previously reported a ratio of 39 based on 2007 data from the James (Bukaveckas et al. 2011). Results from these analyses suggest that C:CHLa ratios needed for the deterministic CHLa model can be accurately determined from either dataset in a given year. However, there is appreciable inter-annual variability in this ratio which suggests that a model sensitivity analyses should be performed.

	2011	2012
Magnitude (µg/L)		
Mean	25.6	24.1
Max	79.7	66.7
Frequency (µg/L)		
1-20	53%	50%
21-40	25%	35%
41-60	10%	13%
61-80	13%	3%
81-100	0%	0%
Duration (days)		
>20 µg/L	173	183
>40 µg/L	82	55
Collections		
N	40	40

Table A1. Magnitude, frequency and duration of algal blooms in the tidal freshwater segment of the James Estuary. Data are for station JMS75.

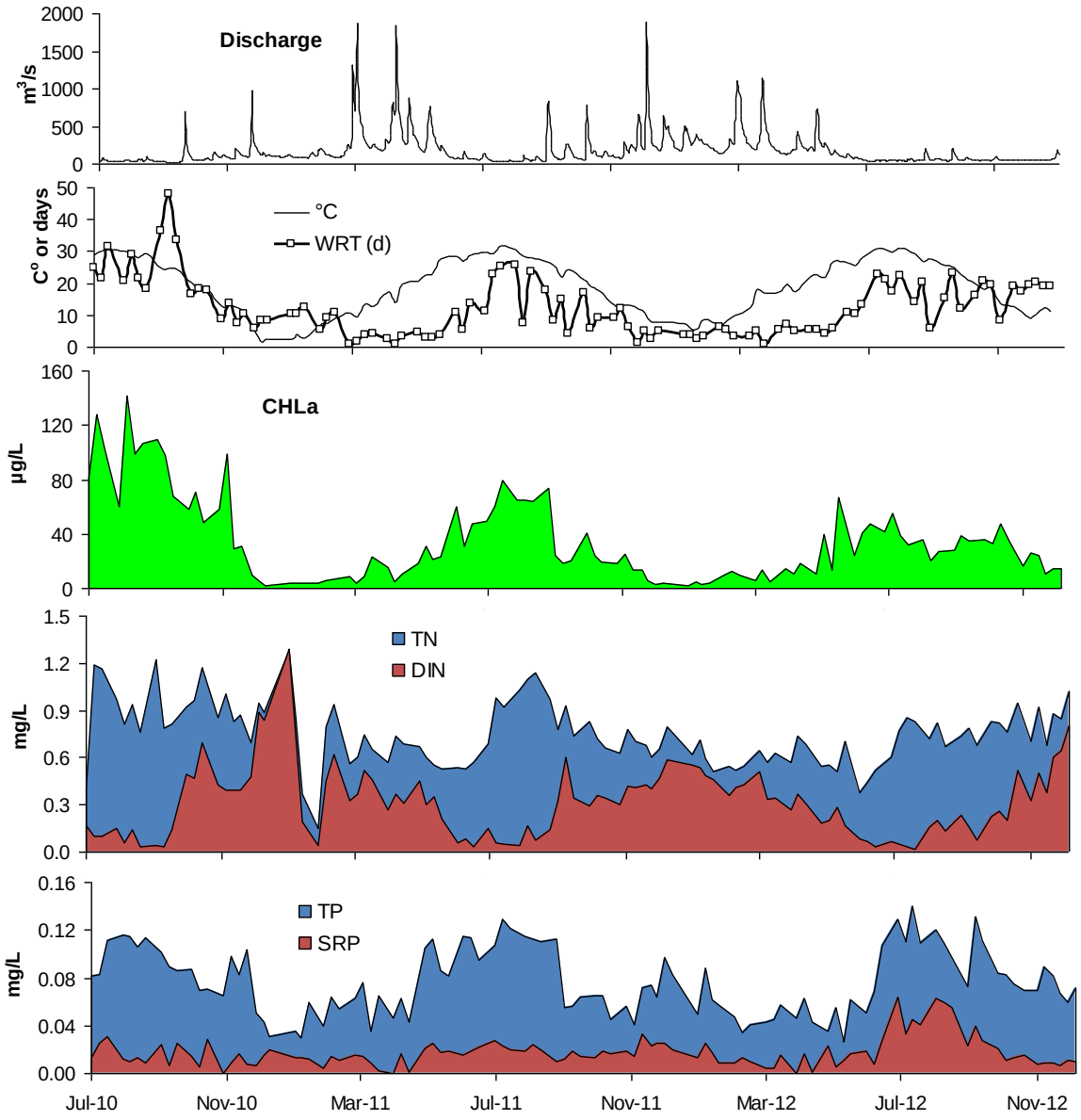


Figure A1. Daily river discharge and weekly water temperature, water residence time (as freshwater replacement), CHLa, TN, DIN, TP and SR P in the tidal freshwater James River at station JMS75 during July 2010 through December 2012.

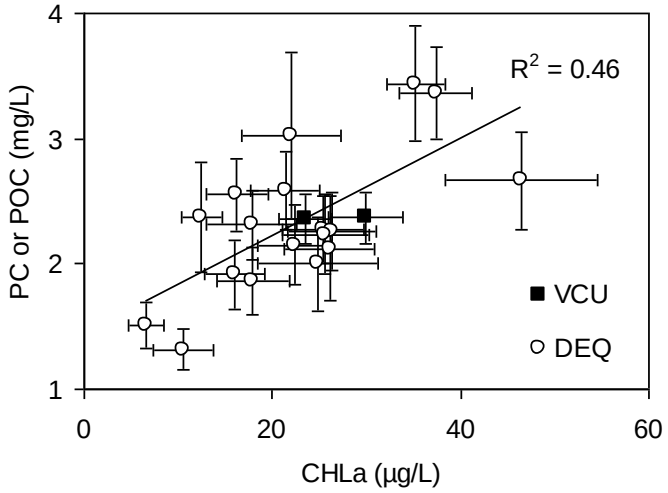


Figure A2. Average concentrations of particulate Carbon and suspended CHLa in the tidal freshwater James River. DEQ data are for 1994-2011; VCU data are 2011 and 2012.

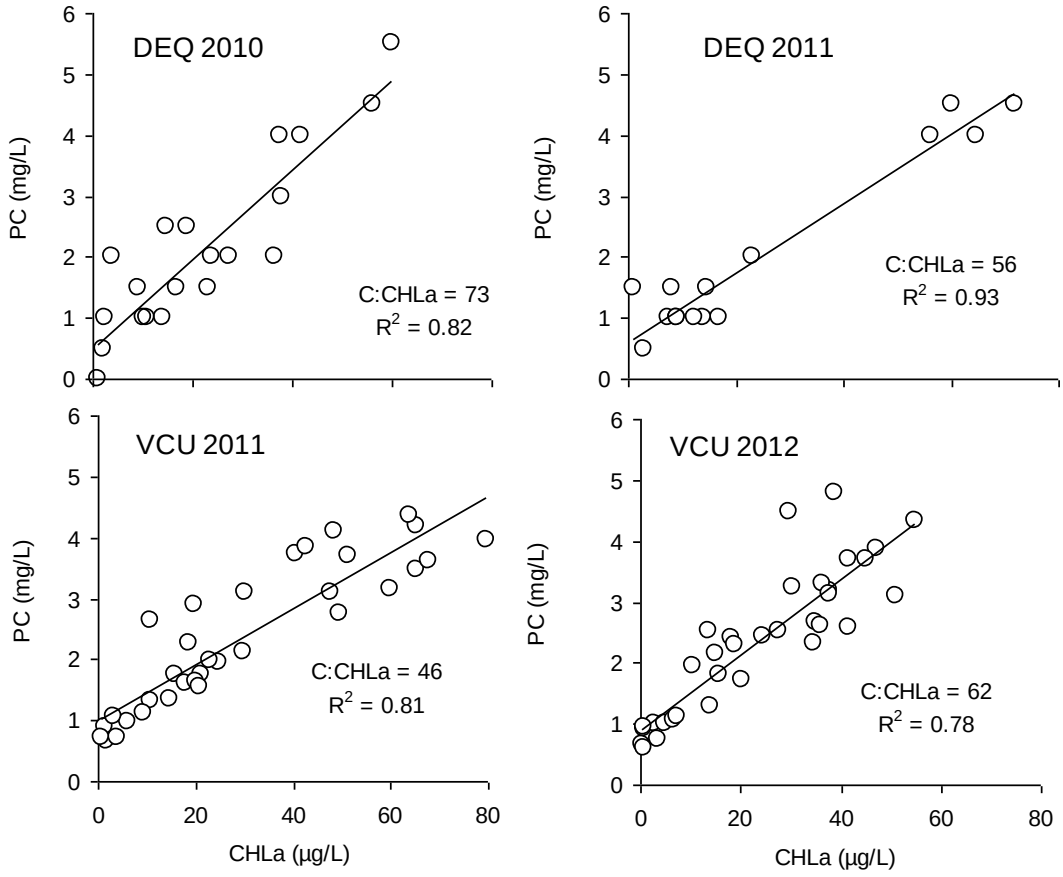


Figure A3. Relationships between CHLa and Particulate Carbon (PC) in monitoring data from the tidal freshwater James River. Datasets for each year are comprised of weekly (VCU) or monthly (DEQ) samples collected from JMS69, 75 and 99 during April-November.

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Supplemental Information (Data Tables)

Table SI-1. Nutrient, CHLa and Microcystin data for JMS75.

Site	Date	[NH3] (mg L^{-1})	[NOx] (mg L^{-1})	[ortho-P] (mg L^{-1})	[TN] (mg L^{-1})	[TP] (mg L^{-1})	CHLa (µg L^{-1})	Microcystin (µg L^{-1})
JMS 75	8-May-12	0.024	0.157	0.023	0.544	0.035	10.3	0.03
JMS 75	15-May-12	0.036	0.165	0.006	0.551	0.055	39.7	0.05
JMS 75	22-May-12	0.053	0.235	0.011	0.514	0.026	13.9	0.02
JMS 75	29-May-12	0.088	0.077	0.016	0.702	0.062	66.7	0.05
JMS 75	6/12/2012	0.068	0.020	0.019	0.379	0.051	24.4	0.08
JMS 75	6/19/2012	0.057	0.011	0.008	0.443	0.068	40.9	0.06
JMS 75	6/26/2012	0.031	0.005	0.027	0.522	0.107	47.1	0.53
JMS 75	7/10/2012	0.052	0.014	0.064	0.604	0.129	41.5	0.60
JMS 75	7/17/2012	0.047	0.004	0.033	0.773	0.111	55.2	0.42
JMS 75	7/24/2012	0.014	0.021	0.045	0.857	0.140	38.8	0.59
JMS 75	7/31/2012	0.009	0.008	0.041	0.827	0.109	31.6	0.37
JMS 75	8/14/2012	0.083	0.078	0.063	0.721	0.120	35.9	0.33
JMS 75	8/21/2012	0.130	0.067	0.059	0.817	0.109	20.1	0.94
JMS 75	8/28/2012	0.016	0.119	0.055	0.671	0.098	27.5	0.47
JMS 75	9/11/2012	0.139	0.100	0.023	0.735	0.073	28.3	0.45
JMS 75	9/18/2012	0.121	0.037	0.039	0.785	0.131	39.0	0.43
JMS 75	9/25/2012	0.043	0.034	0.027	0.679	0.112	34.5	0.25
JMS 75	10/9/2012	0.118	0.106	0.020	0.832	0.084	36.1	0.17
JMS 75	10/16/2012	0.145	0.113	0.011	0.823	0.083	32.6	0.12
JMS 75	10/23/2012	0.155	0.046	0.014	0.763	0.075	47.4	0.17
JMS 75	11/1/2012	0.379	0.144	0.015	0.945	0.069	33.8	0.08
JMS 75	11/13/2012	0.053	0.270	0.008	0.704	0.069	16.9	0.00
JMS 75	11/20/2012	0.192	0.308	0.009	0.921	0.089	26.3	0.00
JMS 75	11/28/2012	0.137	0.242	0.009	0.679	0.081	24.7	0.00

Table SI-2. Nutrient, CHLa and Microcystin data for JMS69.

Site	Date	[NH3] ($mg\ L^{-1}$)	[NOx] ($mg\ L^{-1}$)	[ortho-P] ($mg\ L^{-1}$)	[TN] ($mg\ L^{-1}$)	[TP] ($mg\ L^{-1}$)	CHLa ($\mu g\ L^{-1}$)	Microcystin ($\mu g\ L^{-1}$)
JMS 69	8-May-12	0.037	0.059	0.007	0.419	0.024	20.1	0.05
JMS 69	15-May-12	0.029	0.111	0.001	0.438	0.052	26.8	0.08
JMS 69	22-May-12	0.028	0.058	0.018	0.487	0.020	34.5	0.03
JMS 69	29-May-12	0.018	0.015	0.020	0.509	0.038	50.7	0.07
JMS 69	12-Jun-12	0.049	0.010	0.008	0.372	0.044	34.9	0.14
JMS 69	19-Jun-12	0.033	0.008	0.012	0.360	0.060	21.3	0.19
JMS 69	26-Jun-12	0.023	0.005	0.025	0.413	0.098	30.3	0.40
JMS 69	10-Jul-12	0.074	0.012	0.040	0.504	0.085	37.6	0.74
JMS 69	17-Jul-12	0.041	0.004	0.036	0.859	0.130	54.7	1.24
JMS 69	24-Jul-12	0.001	0.008	0.048	0.630	0.113	54.8	0.67
JMS 69	31-Jul-12	0.028	0.008	0.033	0.865	0.123	38.3	0.53
JMS 69	14-Aug-12	0.064	0.044	0.058	0.710	0.094	51.1	0.52
JMS 69	21-Aug-12	0.065	0.081	0.067	0.664	0.109	22.0	0.52
JMS 69	28-Aug-12	0.030	0.119	0.067	0.932	0.134	36.2	1.10
JMS 69	11-Sep-12	0.078	0.013	0.019	0.618	0.082	37.6	0.64
JMS 69	18-Sep-12	0.088	0.021	0.038	0.592	0.094	26.8	0.29
JMS 69	25-Sep-12	0.064	0.010	0.029	0.736	0.112	45.0	0.69
JMS 69	9-Oct-12	0.218	0.070	0.021	0.614	0.068	19.0	0.20
JMS 69	16-Oct-12	0.070	0.058	0.012	0.698	0.088	27.8	0.12
JMS 69	23-Oct-12	0.133	0.042	0.013	0.756	0.081	29.9	0.13
JMS 69	1-Nov-12	0.257	0.146	0.030	0.804	0.080	25.3	0.09
JMS 69	13-Nov-12	0.142	0.285	0.009	0.849	0.089	22.9	0.06
JMS 69	20-Nov-12	0.093	0.293	0.007	0.756	0.078	20.2	0.00
JMS 69	28-Nov-12	0.141	0.312	0.014	0.796	0.074	16.3	0.00

Table SI-3. Nutrient, CHLa and Microcystin data for JMS56.

Site	Date	[NH3] ($mg\ L^{-1}$)	[NOx] ($mg\ L^{-1}$)	[ortho-P] ($mg\ L^{-1}$)	[TN] ($mg\ L^{-1}$)	[TP] ($mg\ L^{-1}$)	CHLa ($\mu g\ L^{-1}$)	Microcystin ($\mu g\ L^{-1}$)
JMS 56	8-May-12	0.050	0.096	0.011	0.424	0.023	6.3	0.04
JMS 56	15-May-12	0.028	0.127	0.009	0.400	0.040	8.4	0.03
JMS 56	22-May-12	0.021	0.156	0.010	0.334	0.026	22.4	0.04
JMS 56	29-May-12	0.024	0.012	0.020	0.345	0.029	22.9	0.06
JMS 56	12-Jun-12	0.053	0.053	0.012	0.336	0.044	21.2	0.06
JMS 56	19-Jun-12	0.082	0.017	0.022	0.365	0.077	17.8	0.17
JMS 56	26-Jun-12	0.017	0.006	0.031	0.350	0.085	21.0	0.29
JMS 56	10-Jul-12	0.080	0.016	0.037	0.376	0.063	26.6	0.33
JMS 56	17-Jul-12	0.050	0.017	0.044	0.605	0.105	30.5	1.10
JMS 56	24-Jul-12	0.030	0.033	0.055	0.399	0.087	29.7	0.68
JMS 56	31-Jul-12	0.048	0.017	0.032	0.582	0.091	14.7	0.41
JMS 56	14-Aug-12	0.071	0.115	0.066	0.577	0.093	17.6	0.20
JMS 56	21-Aug-12	0.072	0.118	0.063	0.593	0.093	7.6	0.26
JMS 56	28-Aug-12	0.104	0.110	0.069	0.652	0.109	24.8	0.77
JMS 56	11-Sep-12	0.121	0.101	0.036	0.544	0.067	19.0	0.25
JMS 56	18-Sep-12	0.132	0.118	0.039	0.482	0.060	12.7	0.07
JMS 56	25-Sep-12	0.071	0.075	0.029	0.599	0.074	14.4	0.27
JMS 56	9-Oct-12	0.077	0.225	0.052	0.628	0.045	2.8	0.06
JMS 56	16-Oct-12	0.143	0.220	0.013	0.726	0.061	6.2	0.04
JMS 56	23-Oct-12	0.159	0.230	0.022	0.687	0.040	7.9	0.09
JMS 56	1-Nov-12	0.386	0.239	0.040	0.802	0.070	5.5	0.09
JMS 56	13-Nov-12	0.150	0.261	0.009	0.722	0.069	12.8	0.04
JMS 56	20-Nov-12	0.176	0.315	0.020	0.708	0.068	4.4	0.02
JMS 56	28-Nov-12	0.145	0.350	0.021	0.672	0.057	4.2	0.00

Table SI-4. Nutrient, CHLa and Microcystin data for APP1.53.

Site	Date	[NH3] ($mg\ L^{-1}$)	[NOx] ($mg\ L^{-1}$)	[ortho-P] ($mg\ L^{-1}$)	[TN] ($mg\ L^{-1}$)	[TP] ($mg\ L^{-1}$)	CHLa ($\mu g\ L^{-1}$)	Microcystin ($\mu g\ L^{-1}$)
App1.5	8-May-12	0.023	0.174	0.020	0.514	0.024	19.95	0.04
App1.5	15-May-12	0.023	0.184	0.008	0.569	0.048	30.86	0.03
App1.5	22-May-12	0.024	0.043	0.013	0.878	0.132	15.23	0.04
App1.5	29-May-12	0.022	0.137	0.020	0.590	0.052	41.32	0.01
App1.5	12-Jun-12	0.148	0.016	0.017	0.324	0.056	36.94	0.06
App1.5	19-Jun-12	0.026	0.008	0.013	0.374	0.061	32.76	0.13
App1.5	26-Jun-12	0.038	0.004	0.018	0.524	0.099	48.73	0.24
App1.5	10-Jul-12	0.085	0.020	0.057	0.649	0.090	44.45	0.52
App1.5	17-Jul-12	0.056	0.006	0.039	0.738	0.102	46.70	0.23
App1.5	24-Jul-12	0.011	0.071	0.035	0.745	0.084	30.25	0.25
App1.5	31-Jul-12	0.026	0.061	0.047	0.668	0.090	31.66	0.35
App1.5	14-Aug-12	0.029	0.014	0.058	0.651	0.070	32.80	0.25
App1.5	21-Aug-12	0.175	0.078	0.064	0.796	0.104	36.38	0.65
App1.5	28-Aug-12	0.010	0.036	0.054	0.700	0.107	26.22	0.39
App1.5	11-Sep-12	0.083	0.083	0.027	0.711	0.112	22.92	0.24
App1.5	18-Sep-12	0.155	0.062	0.022	0.685	0.084	21.98	0.44
App1.5	25-Sep-12	0.057	0.017	0.023	0.662	0.086	29.76	0.16
App1.5	9-Oct-12	0.117	0.108	0.020	0.625	0.056	24.98	0.18
App1.5	16-Oct-12	0.086	0.123	0.020	0.811	0.088	28.64	0.09
App1.5	23-Oct-12	0.116	0.064	0.014	0.691	0.067	46.82	0.13
App1.5	1-Nov-12	0.216	0.180	0.009	0.814	0.069	28.54	0.05

Table SI-5. Monthly mean concentrations of Microcystin per unit dry and wet weight of liver/viscera and muscle tissues in fish and shellfish from the James River during 2012.

	Liver/ Viscera (µg/g dry weight)							Muscle Microcystin (µg/g dry weight)						
	24-May-12	28-Jun-12	26-Jul-12	30-Aug-12	27-Sep-12	25-Oct-12	Mean	24-May-12	28-Jun-12	26-Jul-12	30-Aug-12	27-Sep-12	25-Oct-12	Mean
Blue Catfish (<20 cm)	0.260	0.049	0.040	0.040	0.039	nd	0.086	0.0000	0.0000	0.0050	0.0000	0.0001	nd	0.0010
Blue Catfish (20-40 cm)	0.265	0.068	0.044	0.029	0.146	0.006	0.093	0.0007	0.0000	0.0104	0.0010	0.0006	0.0000	0.0021
Blue Catfish (>40 cm)	0.013	0.021	0.028	0.034	0.031	0.030	0.026	0.0000	0.0004	0.0025	0.0000	0.0001	0.0000	0.0005
Gizzard Shad	0.033	0.030	0.065	0.061	0.030	0.013	0.039	0.0048	0.0000	0.0011	0.0047	0.0025	0.0004	0.0022
Gizzard Shad YOY	nd	0.987	0.093	0.188	0.083	0.111	0.292	nd	0.0013	0.0143	0.0016	0.0020	0.0000	0.0038
Threadfin Shad	nd	0.006	0.265	0.330	0.173	0.052	0.165	nd	0.0000	0.0097	0.0066	0.0006	0.0000	0.0034
Atlantic Menhaden	0.180	nd	nd	nd	0.136	0.146	0.154	0.0000	nd	nd	nd	0.0061	0.0076	0.0046
Rangia	0.016	0.014	0.030	0.033	0.037	0.011	0.023	0.0003	0.0005	0.0057	0.0172	0.0193	0.0007	0.0073
Blue Crab	0.088	nd	0.073	0.232	0.127	0.072	0.118	0.0167	nd	0.0076	0.0372	0.0092	0.0201	0.0182
Mean	0.122	0.168	0.080	0.118	0.089	0.055		0.0032	0.0003	0.0070	0.0086	0.0045	0.0036	

	Liver/ Viscera (µg/g wet weight)							Muscle Microcystin (µg/g wet weight)						
	24-May-12	28-Jun-12	26-Jul-12	30-Aug-12	27-Sep-12	25-Oct-12	Mean	24-May-12	28-Jun-12	26-Jul-12	30-Aug-12	27-Sep-12	25-Oct-12	Mean
Blue Catfish (<20 cm)	0.0840	0.0158	0.0130	0.0128	0.0125	nd	0.0276	0.00000	0.00000	0.00161	0.00000	0.00004	nd	0.00033
Blue Catfish (20-40 cm)	0.0855	0.0221	0.0142	0.0093	0.0471	0.0019	0.0300	0.00023	0.00000	0.00337	0.00034	0.00019	0.00000	0.00069
Blue Catfish (>40 cm)	0.0040	0.0068	0.0091	0.0109	0.0099	0.0096	0.0084	0.00000	0.00012	0.00079	0.00000	0.00004	0.00000	0.00016
Gizzard Shad	0.0105	0.0096	0.0210	0.0197	0.0098	0.0043	0.0125	0.00155	0.00000	0.00035	0.00152	0.00080	0.00013	0.00072
Gizzard Shad YOY	nd	0.3185	0.0299	0.0606	0.0267	0.0359	0.0943	nd	0.00042	0.00460	0.00052	0.00064	0.00000	0.00123
Threadfin Shad	nd	0.0019	0.0854	0.1063	0.0558	0.0168	0.0533	nd	0.00000	0.00313	0.00213	0.00018	0.00000	0.00109
Atlantic Menhaden	0.0581	nd	nd	nd	0.0438	0.0472	0.0497	0.00000	nd	nd	nd	0.00198	0.00244	0.00147
Rangia	0.0052	0.0046	0.0097	0.0106	0.0118	0.0034	0.0076	0.00011	0.00016	0.00183	0.00556	0.00624	0.00022	0.00235
Blue Crab	0.0282	nd	0.0235	0.0748	0.0411	0.0232	0.0382	0.00538	nd	0.00245	0.01201	0.00297	0.00649	0.00586
Mean	0.0394	0.0542	0.0257	0.0381	0.0287	0.0178		0.00104	0.00010	0.00227	0.00276	0.00145	0.00116	